Electronic Supplementary Material (ESI) for MedChemComm.

Supporting Information for:

Anti-bacterial glycosyl triazoles – Identification of an N-acetylglucosamine derivative with bacteriostatic activity against Bacillus

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Synthesis

General Methods

Compounds were purchased from standard suppliers and used without further purification. Thin layer chromatography was carried out on Merck silica gel 60 F_{254} precoated glass plates. Spots were detected by UV light and immersion in *p*-anisaldehyde stain or potassium permanganate stain. Column chromatography was performed on Teledyne ISCO CombiFlash® Rf purification system using pre-packed RediSep Rf Gold® Normal-Phase Silica Flash cartridges and gradient elution or with DAVISIL® Chromatographic Silica Media LC60A 35-70 micron. Evaporation of solvents was performed under reduced pressure at 30-40 °C. NMR spectra were recorded on Bruker Avance III HD Ascend 600 MHz and Bruker Avance Ultra-Shield 400 MHz. ^{1}H, ^{13}C, COSY, HSQC and ^{13}C-DEPT135 were referenced to residual solvent peaks. The coupling constants are given in [Hz] and chemical shifts in [ppm]. Electrospray ionization (ESI) and fast atom bombardment (FAB) mass spectra were obtained using JEOL JMS-600H double focusing magnetic sector mass spectrometer for HRMS and Thermo LCQ Deca XP Max ion trap mass spectrometer with Shimadzu HPLC system for LRMS in positive mode. The Ugi products as well as the glycosyl triazoles exist as mixtures of rotamers (and diastereomeric mixtures in the case of the triazoles), resulting in multiple peaks in the NMR spectra. Partial assignments are provided.

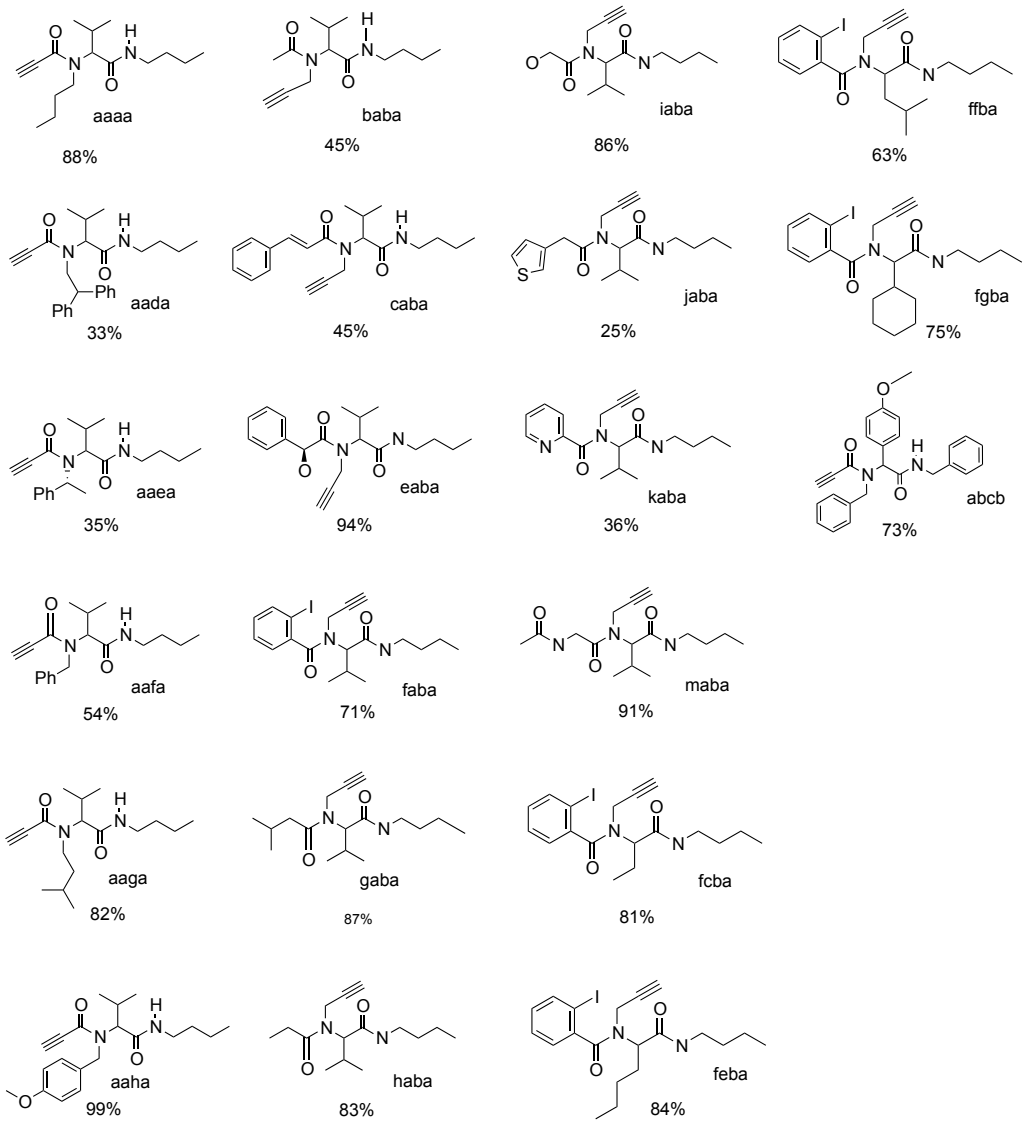
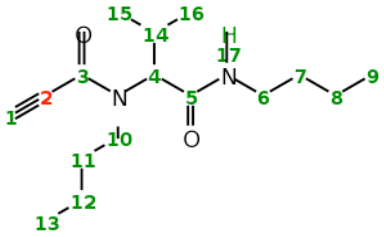


Figure S1 – Structure of Ugi products prepared for GNT synthesis. Percentage values correspond to isolated yields.

aaaa

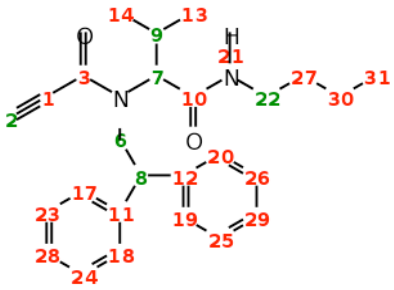


Isobutyraldehyde (0.23 mL, 2.50 mmol) was dissolved in MeOH (2.50 mL) and n-butylamine (0.25 mL, 2.50 mmol) was added and the solution was stirred for 15-20 minutes at room temperature. Propiolic acid (0.15mL, 2.50mmol) followed by n-butylisocyanide (0.26 mL, 2.50mmol) was added to the solution and stirred over night at room temperature. The solvent was evaporated in vacuo and the residue was purified by FC (20:1 DCM/ isopropylalcohol).A white-yellow solid was obtained. Yield 620mg (88 %, 2.00mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ [6.61 (s) **NH minor**; 5.65 (s) **NH major**], [4.21 (d, *J* = 10.6 Hz) **4 minor**; 4.04 (d, *J* = 10.1 Hz) **4 major**, 1H], [3.67 – 3.55 (m,1H); 3.49 (m,1H), **10**], [3.30 – 3.23 (m) **6 minor**; 3.23 – 3.17 (m) **6 major**, 2H], 3.17 (s, 1H, **1**), [2.50 (m)**14 major**; 2.42 – 2.28 (m)**14 minor**, 1H], 1.61 (m, 2H, **11**), 1.52 – 1.39 (m, 2H, **7**), 1.37 – 1.26 (m, 4H, **12**, **8**), 1.00 – 0.79 (m, 12H, **9**, **13**, **15**, **16**)ppm.

ESI-LRMS (*m/z*): calcd. for $C_{16}H_{28}N_2O_2$ + H: 281.22; found 281.57.

aada



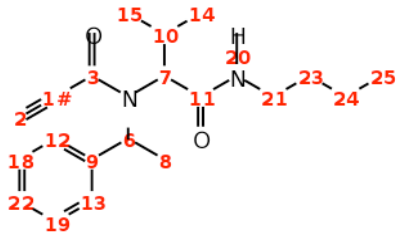
Isobutyraldehyde (0.09 mL, 1.00 mmol) was added under N_2 atmosphere to MeOH (0.25 mL) with molecular sieve. 2,2-Diphenylethylamine (0.20 g, 1.00mmol) was dissolved in MeOH (0.25 mL) and added to the reaction mixture which was stirred at room temperature for 20 minutes. After cooling down the solution to 0°C propiolic acid (0.06 mL, 1.00 mmol) followed by n-butylisocyanide (0.11 mL, 1.00 mmol) were rapidly added to the reaction mixture. The reaction was stirred at room temperature and tested by TLC for completion. Molecular sieves were filtered off through Celite which were rinsed with diethylether and then methanol. The solvent was evaporated in vacuo and the residue was washed with hexane. The residue was dispersed in MeOH and addition of water lead to product precipitation, which was filtered off and dried under high vacuum. A white-yellow solid was obtained. Yield 130mg (33 %, 0.33 mmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.34 – 7.14 (m, 11H, **aromatic, 21**), [4.78 – 4.67 (m) **6 minor**; 4.65 – 4.47 (m) **6 major**, 2H], [4.21 – 4.07 (m) **8 minor**; 4.05 – 3.88 (m) **8 major**, 1H], 3.65 (d, *J*=11.3, 1H, **7**), 3.21 – 2.99 (m, 2H, **22**), [2.74 – 2.62 (m) **9 major**; 2.44 – 2.29 (m) **9 minor**;

1H], 1.65 (s, 1H, **2**), 1.42 – 1.18 (m, 4H, **27, 30**), [0.97 (d, *J*=6.5) **13 major**; 0.94 (d, *J*=6.5) **13 minor**, 3H], 0.88 (t, *J*=7.2, 3H, **31**), [0.83 (d, *J*=6.5) **14 major**; 0.68 (d, *J*=6.6) **14 minor**, 3H] ppm.

ESI-LRMS (*m/z*): calcd. for $C_{26}H_{32}N_2O_2$ + H: 405.25; found 405.84.

aaea



(S)-(-)-1-phenylethylamine (0.38 mL, 3.00 mmol, 1.8 eq.) and isobutyraldehyde (0.18 mL, 2.00 mmol, 1.2 eq) were added to MeOH (4.00 mL) at room temperature and stirred for 10 minutes. Propiolic acid (0.12 mL, 2.00 mmol, 1.2 eq.) was added and the reaction was stirred for 5 min. at room temperature, followed by addition of n-butylisocyanide (0.18 mL, 1.71 mmol, 1.0 eq.) and stirring at room temperature until completion was shown by TLC. The reaction mixture was extracted two times with hexane, the solvent was evaporated in vacuo and the residue was dispersed in ethylactetate. The organic layer was extracted with 1 M HCl, H_2O, saturated $NaHCO_3$, H_2O and brine (2x). The organic layer was dried with $MgSO_4$, the solvent was evaporated in vacuo and the residue was dried under high vacuum. The product was further purified by flash chromatography and gradient elution using DCM and isopropylalcohol. A clear syrup was obtained. Yield 190mg (35 %, 0.59 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (s, **20**), [7.53 – 7.42 (m); 7.36 (m), 5H, **aromatic]**, 5.94 (q, *J* = 7.1 Hz, 1H, **6**), 3.37 (s, 1H, **2**), 3.27 – 3.16 (m, 2H, **21**), 3.10 (d, *J* = 11.1 Hz, 1H, **7**), 2.81 – 2.64 (m, 1H, **10**), 1.66 (d, *J* = 7.2 Hz, 3H, **8**), 1.52 – 1.40 (m, 2H-**23**), 1.38 – 1.27 (m, 2H-**24**), 0.92 (t, *J*=7.3, 3H, **25**), 0.72 (d, *J* = 6.6 Hz, 3H, **14**), 0.13 – 0.01 (m, 3H, **15**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{20}H_{28}N_2O_2$ + H: 329.22; found 329.87.

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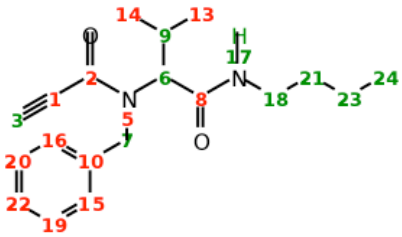


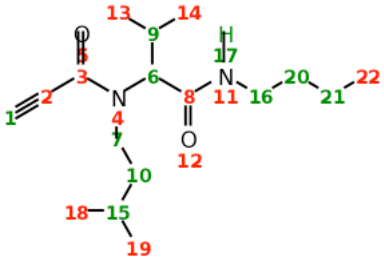
Isobutyraldehyde (0.18 mL, 2.00 mmol, 1.0 eq.) was added to a solution of benzylamine (0.44 mL, 4.00 mmol, 2.00 eq.) in MeOH (4.00 mL) at room temperature and stirred for 10 minutes. Propiolic acid (0.13 mL, 2.00 mmol, 1.0 eq.) was added and the reaction was stirred for 5 min. at room temperature followed by addition of n-butylisocyanide (0.34 mL, 3.20 mmol, 1.6 eq.) and stirring at room temperature until completion was shown by TLC. The reaction mixture was

extracted two times with hexane, the solvent was evaporated in vacuo and the residue was dispersed in ethylacetate. The organic layer was extracted 1 M HCl, H_2O, saturated $NaHCO_3$, H_2O and brine (2x). The organic layer was dried with $MgSO_4$, the solvent was evaporated in vacuo and the residue was dried under high vacuum. The product was further purified by flash chromatography and gradient elution using DCM and isopropylalcohol. and a white-yellow syrup was obtained. Yield 30mg (54 %, 1.08 mmol). R_f (20:1 DCM/isopropylalcohol): 0.6.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.16 (m, 5H, **aromatic**), [6.49 (s) **NH major**; 5.52 (s**) NH minor**], [4.99 (d, *J* = 16.0 Hz) **7 major**; 4.76 (d, *J*=14.7) **7 minor**; 4.73 (d, *J*=16.0) **7 major**; 4.55 (d, *J*=14.8) **7 minor**, 1H], [4.32 (d, *J* = 10.6 Hz) **6 minor**; 4.15 – 3.99 (m) **6 major**, 1H], [3.28 (s) **3 minor**; 3.20 (s) **3 major**, 1H], 3.16 – 2.96 (m, 2H, **18**), 2.55 – 2.37 (m, 1H, **9**), 1.44 – 1.33 (m, 2H, **21**), 1.33 – 1.18 (m, 2H, **23**), [0.97 (d, *J*=6.4) **13 minor**; 0.93 – 0.81 (m) **13 major**, 3H], 0.93 – 0.81 (m, 3H, **24**), [0.77 (d, *J*=6.7) **14 minor**; 0.65 (d, *J*=6.6) **14 major**, 3H] ppm.

ESI-LRMS (*m/z*): calcd. for $C_{19}H_{26}N_2O_2$ + H: 315.21; found 315.61.

aaga

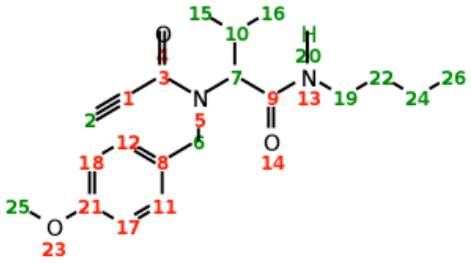


Isoamylamine (0.23 mL, 2.00 mmol, 1.0 eq.) was dissolved in MeOH (2.00 mL), isobutyraldehyde (0.27 mL, 3.00 mmol, 1.5 eq.) was added and the solution was stirred at room temperature for 10 minutes. Propiolic acid (0.13 mL, 2.00 mmol, 10.0 eq.) was added and the reaction mixture was stirred for 5 minutes, followed by addition of n-butylisocyanide (0.32 mL, 3.00 mmol, 1.5 eq.). The reaction mixture was stirred at room temperature until TLC showed completion. The methanolic solution was extracted 3x with hexane and the solvent was evaporated in vacuo. The residue was dispersed in EtOAc (15.00 mL) and the organic layer was extracted 3x H_2O and 2x brine, the solvent was removed and the residue was purified by FC (20:1 DCM/ isopropylalcohol). A white-yellow syrup was obtained. Yield 480mg (82 %, 1.63 mmol).

^{1}H NMR (400 MHz, Acetone-d_6) δ [7.40 (s) **NH minor**; 7.28 (s) **NH major**], [4.40 (d, *J*=10.6) **6 minor**; 4.34 (d, *J*=11.0) **6 major,** 1H], [3.96 (s) **1 major**; 3.86 (s) **1 minor**, 1H], [3.80 – 3.63 (m); 3.61 – 3.42 (m); 3.36 – 3.08 (m), 2H**, 7]**, [3.36 – 3.08 (m), 2H**, 16],** 2.46 – 2.22 (m, 1H, **9**), 1.69 – 1.21 (m, 7H, **10, 15, 20, 21**), 1.01 – 0.74 (m, 15H, **13**, **14**, **18**, **19**, **22**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{17}H_{30}N_2O_2$ + H: 295.24; found 295.76.

aaha

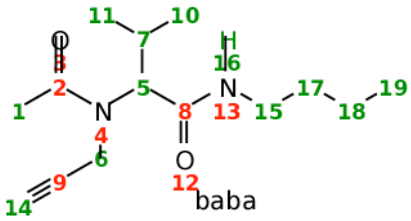


To a solution of 4-methoxybenzylamine (10.00mL of 0.1M in MeOH, 2.00mmol) isobutyraldehyde (10.00mL of 0.1M in MeOH, 2.00mmol) was added and the solution was stirred for 30 min. at 40°C. *N*-butylisocyanide (10.00mL of 0.1M in MeOH, 2.00mmol) was added to the solution and stirred for 20 min. at 50°C. After addition of propiolic acid (10.00mL of 0.1M in MeOH, 2.00mmol) the reaction mixture was reflux for 3h. The solvent was evaporated in vacuo and the residue was dried under high vacuo at 40°C in a water-bath. For purification the residue was dissolved in MeOH and treated with activated charcoal and filtered off over Celite, the solvent was evaporated in vacuo and the residue was dried under high vacuum. A yellow syrup was obtained. Yield 680mg (99%, 1.97 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ = [7.35 (s); 7.32 – 7.27 (m); 6.90 – 6.80 (m); 6.81 – 6.73 (m), 4H, **aromatic**], 6.60 (s, **NH-20**), [4.93 (d, *J*=15.7) **6 major**; 4.65 (d, *J*=15.7) **6 major**; 4.49 (d, *J*=14.5) **6 minor**, 2H], [4.30 (d, *J*=10.6) **7 minor**; 4.01 (d, *J*=11.1) **7 major**, 1H], [3.78 (s) **25 major**; 3.75 (s) **25 minor**, 3H], [3.27 (s) **2 minor**; 3.23 (s) **2 major**, 1H], 3.18 – 2.92 (m, 2H, **19**), 2.56 – 2.38 (m, 1H, **10**), 1.42 – 1.32 (m, 2H, **22**), 1.32 – 1.18 (m, 2H, **24**), [0.96 (d, *J*=6.4) **15minor**; 0.94 – 0.82 (m) **15 major**, 3H], 0.94 – 0.82 (m, 3H, **26**), 0.75 (d, *J*=6.7) **16 minor**; 0.62 (d, *J*=6.6) **16 major**, 3H] ppm.

ESI-LRMS (*m/z*): calcd. for $C_{20}H_{28}N_2O_3$ + H: 345.22; found 345.48.

baba



Propargylamine (0.13 mL, 2.00 mmol) was dissolved in MeOH (0.50 mL) and isobutyraldehyde (0.18 mL, 2.00 mmol) was added and the solution was stirred for 15-20 minutes at room temperature. The solution was cooled down to 0°C and MeOH (0.50 mL), followed by addition of propiolic acid (0.11 mL, 2.00 mmol) and n-butylisocyanide (0.25 mL, 2.00 mmol). The reaction mixture was stirred at room temperature until TLC showed completion. The methanolic solution was extracted 3x with hexane and the solvent was evaporated in vacuo. The residue was dissolved in water leading to a formation of white-yellow solid which was filtered off and rinsed with H_2O. The residue was dried under high vacuum. Yield 180mg (45%, 0.71mmol). R_f (20:1 DCM/isopropylalcohol): 0.59.

^{1}H NMR (400 MHz, Acetone-d_6) δ = [7.49 (s) **NH minor**; 7.25 (s) **NH major**], [4.70 – 4.51 (m);

3.88 (d, *J*=10.6) **5 major**; 3.88 (d, *J*=10.6) **5 minor**, 1H], [4.70 – 4.51 (m); 4.48 – 4.35 (m); 4.28 – 4.08 (m), 2H, **6]**, [3.32 – 3.17 (m); 3.17 – 3.05 (m), 1H, **15]**, 2.89 (s, 1H, **14**), [2.40 – 2.30 (m) **7 minor**; 2.29 – 2.14 (m) **7 major**, 1H], [2.29 – 2.14 (m); 2.12 – 1.99 (m), 1H, **1]**, 1.54 – 1.42 (m, 2H, **17**), 1.40 – 1.26 (m, 2H, **18**), 1.12 – 0.68 (m, 9H, **10, 11, 19**) ppm.

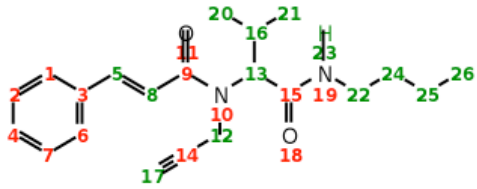
ESI-LRMS (*m/z*): calcd. for $C_{14}H_{24}N_2O_2$ + H: 253.19; found 253.70.

Aglycone synthesis using optimized conditions: c-m.a-g.b-c.a

General procedure for c-m.a-g.b-c.a

Of each reactant- amine, aldehyde, acid and isocyanide- a 0.1 M solution in MeOH was prepared. Amine (5.00 mL of 0.1 M) and aldehyde (5.00 mL of 0.1 M) were stirred in a capped scintillation vial for 30 min. at 35-40°C. Then isocyanide (5.00 mL of 0.1 M) was added to this solution and the reaction mixture was stirred for additional 20 minutes at 50°C. Lastly the acid (5.00 mL of 0.1 M) was added and the reaction mixture was continuously stirred at 55°C for 3-5 h . The progress of the reaction was monitored using UV-light, *p*-anisaldehyde and potassium permanganate for visualization. When the reaction was finished the solvent was removed in vacuo and the crude product was dried under high vacuum. When the reaction ran to completion according to the TLC, an NMR and ESI-LRMS was obtained of the crude product and depending on purity, the crude product was either pure enough for the next reaction or it was purified using a silica and/or basic alumina plug. In case of incomplete reaction the solvent was removed in vacuo and the residue was dried under high vacuum for at least 30 minutes to ensure that all methanol was evaporated. The dried residue was dissolved in a minimum amount of solvent and purified using a silica or basic alumina plug. The most common solvents used for the silica plug were 20:1 DCM/MeOH, 20:1 DCM/EtOAc and 40:1 DCM/EtOAc. After purification the fractions of interest were tested by MS-ESI, combined and after the removal of solvent, the product was dried under high vacuum and analyzed by NMR.

caba



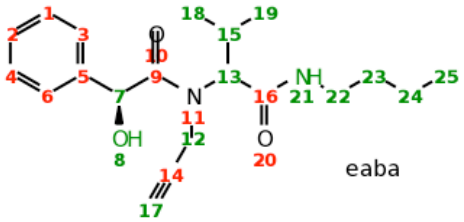
Yield: 77mg (45%, 0.23 mmol), white-yellow solid

R_f (20:1 DCM/isopropylalcohol): 0.59

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.74 (d, *J*=15.4, 1H, **5**), [7.62 – 7.49 (m); 7.45 – 7.31 (m), 5H, **aromatic**], 7.06 (d, *J*=15.4, 1H, **8**), 6.32 (s, **NH-23**), [4.63 – 4.51 (m); 4.16 (d, *J*=2.5); 4.11 (d, *J*=2.5), 3H, **12, 13**], 3.32 – 3.08 (m, 2H, **22**), 2.45 – 2.28 (m, 2H, **16, 17**), 1.52 – 1.37 (m, 2H, **24**), 1.31 (m, 2H, **25**), 1.00 (d, *J*=3.5, 3H, **20**), 0.98 (d, *J*=3.7, 3H, **21**), 0.88 (t, *J*=7.3, 3H, **26**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{21}H_{28}N_2O_2$ + H: 341.22; found 341.82.

eaba



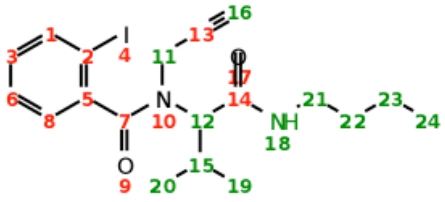
Yield: 160mg (94%, 0.47 mmol), yellow syrup

R_f (20:1 DCM/isopropylalcohol): 0.57

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.52 – 7.27 (m, 5H, **aromatic**), 6.19 – 5.97 (m, **NH-21**), 5.62 – 5.49 (m, 1H, **7 or 8**); [4.53 – 4.48 (m); 4.43 – 4.38 (m), 1H, **7 or 8**)], [4.55 (d, *J*=11.1); 4.47 (d, *J*=11.1); 3.42 (d, *J*=10.7), 1H, **13]**, [4.42 (d, *J*=2.7); 4.38 (d, *J*=1.2); 4.33 (d, *J*=2.6); 4.18 (d, *J*=2.5); 4.13 (d, *J*=2.4); 3.96 (d, *J*=2.5); 3.91 (d, *J*=2.5); 3.83 (d, *J*=2.5); 3.78 (d, *J*=2.5); 3.63 (d, *J*=2.5); 3.58 (d, *J*=2.5), 2H, **12]**, [3.31 – 3.01 (m); 2.93 – 2.83 (m); 2.66 – 2.55 (m), 2H, **22**], [2.41 (t, *J*=2.5); 2.25 (t, *J*=2.4); 2.16 (t, *J*=2.5); 1H, **17]**, [2.38 – 2.26 (m); 2.23 – 2.17 (m), 1H, **15]**, 1.56 – 1.21 (m, 4H, **23, 24**), 1.13 – 0.48 (m, 9H, **18**, **19**, **25**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{20}H_{28}N_2O_3$ + H: 345.22; found 345.47.

faba



Yield: 160mg (71%, 0.36 mmol), white-yellow solid

R_f (20:1 DCM/isopropylalcohol): 0.66

^{1}H NMR (400 MHz, Acetone-d_6) δ [7.97 – 7.82 (m); 7.56 – 7.44 (m); 7.44 – 7.27 (m); 7.25 – 7.08 (m), 5H, **aromatic, NH-18]**, [5.02 (d, *J*=2.5); 4.98 (d, *J*=2.6); 4.89 – 4.75 (m); 4.76 – 4.62 (m); 4.56 (d, *J*=2.6); 4.54 (d, *J*=2.5); 4.52 (d, *J*=2.5); 4.50 (d, *J*=2.5), 2H, **11]**, [3.65 (d, *J*=10.4) **12 major**; 3.59 (d, *J*=10.0) **12 minor**, 1H], [3.34 – 3.13 (m) **21 major**; 3.14 – 3.03 (m) **21 minor**, 2H], [2.66 (t, *J*=2.5) **16 major**; 2.62 (t, *J*=2.5) **16 minor**; 2.60 (t, *J*=2.5) **16 minor**, 1H], 2.51 – 2.30 (m, 1H, **15**), 1.58 – 1.39 (m, 2H, **22**), 1.42 – 1.23 (m, 2H, **23**), 1.19 – 1.04 (m, 3H, **19**), [1.00 (d, *J* = 6.5 Hz) **20 major**; 0.96 – 0.78 (m) **20 minor**, 3H], 0.96 – 0.78 (m, 3H, **24**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{19}H_{25}IN_2O_2$ + H: 441.10; found 441.40.

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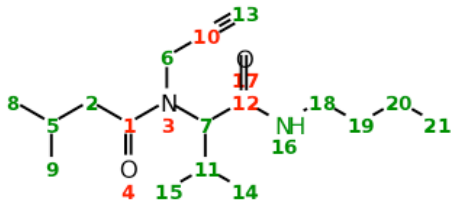


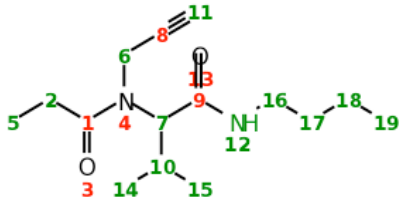
Yield: 128mg (87%, 0.44 mmol), yellow solid

R_f (20:1DCM/isopropylalcohol): 0.63

^{1}H NMR (400 MHz, Acetone-d_6) δ [7.40 (s) **NH minor**; 7.12 (s) **NH major**], 4.64 – 4.59 (m, 1H, **7**), [4.59 – 4.54 (m); 4.48 (d, *J*=2.5); 4.44 (d, *J*=2.4); 4.29 (d, *J*=2.4); 4.25 (d, *J*=2.4); 4.14 (d, *J*=2.5); 4.09 (d, *J*=2.4), 2H, **6**], 3.27 – 3.08 (m, 2H, **18**), 2.79 (t, *J* = 2.5 Hz, 1H, **13**), 2.49 – 2.31 (m, 2H, **2**), 2.29 – 2.11 (m, 2H, **5, 11**), 1.46 (m, 2H, **19**), 1.39 – 1.25 (m, 2H, **20**), 1.04 – 0.84 (m, 15H, **8, 9, 14, 15, 21**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{17}H_{30}N_2O_2$ + H: 295.24; found 295.78.

haba



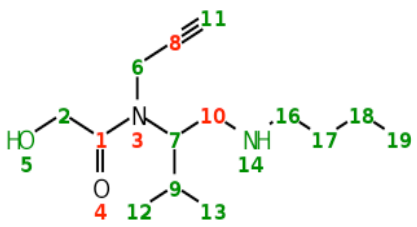
Yield: 111mg (83%, 0.42 mmol), yellow solid

R_f (20:1 DCM/isopropylalcohol): 0.65

^{1}H NMR (400 MHz, Acetone-d_6) δ [7.41 (s) **NH minor**; 7.15 (s) **NH major**], 4.60 – 4.55 (m, 1H, **7**), [4.65 – 4.60 (m); 4.50 – 4.41 (m); 4.31 – 4.20 (m); 4.15 (d, *J*=2.5); 4.10 (d, *J*=2.5), 2H, **6**), 3.28 – 3.06 (m, 2H, **16**), 2.79 (t, *J* = 2.5 Hz, 1H, **11**), 2.65 – 2.43 (m, 2H, **2**), 2.32 – 2.16 (m, 1H, **10**), 1.47 (m, 2H, **17**), 1.39 – 1.25 (m, 2H, **18**), 1.14 – 1.02 (m, 3H, **5**), 1.03 – 0.82 (m, 9H, **14, 15, 19**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{15}H_{26}N_2O_2$ + H: 267.21; found 267.69.

iaba



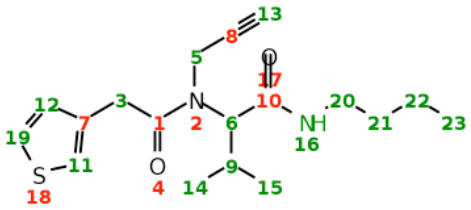
Yield: 115mg (86%, 0.43 mmol), yellow syrup

R_f (20:1 DCM/isopropylalcohol): 0.63

^{1}H NMR (400 MHz, Acetone-d_6) δ [7.45 (s) **NH minor**; 7.34 (s) **NH major**], 4.59 (d, J = 11.0 Hz) **7 major**; 3.65 (d, J=10.5) **7 minor**, 1H], [4.38 (d, J=4.8) **2 major**; 4.34 (d, J=4.8) **2 minor**, 2H], [4.67 (d, J=2.5); 4.62 (d, J=2.5); 4.55 (d, J=2.5); 4.51 (d, J=2.5); 4.31 – 4.27 (m); 4.24 (d, J=2.5); 4.14 (d, J=2.5); 4.09 (d, J=2.5), 2H, **6**], 3.57 (t, J = 4.8 Hz, 1H, **5**), 3.33 – 3.06 (m, 2H, **16**), 2.92 – 2.75 (m, 1H, **11**), [2.45 – 2.32 (m) **9 minor**; 2.32 – 2.17 (m) **9 major**, 1H], 1.47 (m, 2H, **17**), 1.42 – 1.24 (m, 2H, **18**), 1.06 – 0.80 (m, 9H, **12, 13, 19**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{14}H_{24}N_2O_3$ + H: 269.19; found 269.47.

jaba



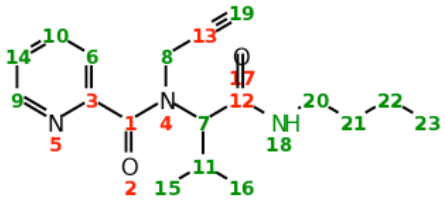
Yield: 42mg (25%, 0.13mmol), yellow syrup

R_f (10:1 DCM/MeOH): 0.83

^{1}H NMR (400 MHz, $CDCl_3$) δ = [7.32 – 7.27 (m); 7.13 – 7.07 (m); 7.04 – 6.97 (m) 3H, **aromatic**], 6.11 (s, **NH-16**), 4.47 (d, J=11.1, 1H, **6**), [4.39 (d, J=2.5); 4.34 (d, J=2.5); 4.01 (d, J=2.5); 3.96 (d, J=2.5), 2H, **5**], 3.94 – 3.91 (m, 2H, **3**), 3.26 – 3.09 (m, 2H, **20**), 2.33 (t, J=2.5, 1H, **13**), 2.32 – 2.22 (m, 1H, **9**), 1.48 – 1.35 (m, 2H, **21**), 1.35 – 1.19 (m, 2H, **22**), 1.04 – 0.78 (m, 9H, **14, 15, 23**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{18}H_{26}N_2O_2S$ + H: 335.18; found 335.70.

kaba

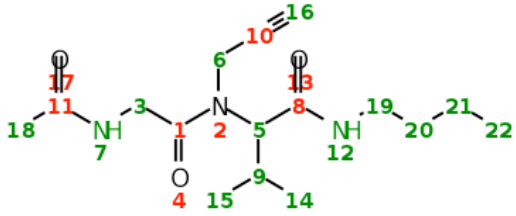


Yield: 57mg (36%, 0.18mmol), yellow syrup

^{1}H NMR (400 MHz, $CDCl_3$) δ [8.95 – 8.76 (m); 8.71 (dd, *J*=4.9, 1.7); 7.99 – 7.84 (m); 7.41 – 7.35 (m), 4H, **aromatic**], 6.58 (s, **NH-18**), 4.38 – 4.26 (m, 1H, **7**), [4.53 – 4.37 (m); 3.98 – 3.91 (m); 3.91 – 3.86 (m), 2H, **8**], 3.29 – 3.21 (m, 2H-**20**), 2.65 – 2.49 (m, 1H-**11**), 2.30 (t, *J* = 2.4 Hz, 1H-**19**), 1.55 – 1.42 (m, 2H-**21**), 1.39 – 1.26 (m, 2H-**22**), 1.08 (d, *J*=6.5, 3H, **15**), 1.02 (d, *J*=6.3, 3H, **16**), 0.92 (t, *J* = 7.3 Hz, 3H, **23**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{18}H_{25}N_3O_2$ + H: 316.20; found 317.79.

maba

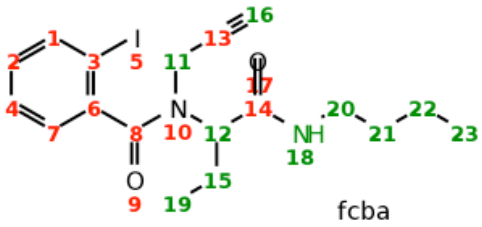


Yield: 142mg (91%, 0.46 mmol), yellow solid

^{1}H NMR (400 MHz, Acetone-d_6) δ (7.80 (s, **NH12-minor),** 7.34 (s, **NH7-minor**), 7.24 (s, **NH12-major**), 7.11 (s, **NH7-major**), [4.58 (d, *J* = 10.9 Hz) **5-major**; 4.00 – 3.93 (m) **5-minor**, 1H], [4.70 (d, *J* = 2.6 Hz); 4.66 (d, *J* = 2.6 Hz**);** 4.49 (d, *J*=2.6); 4.44 (d, *J*=2.6); 4.29 – 4.22 (m); 4.21 (d, *J*=2.5); 3.94 – 3.85(m), 2H, **6**], 4.29 – 4.22 (m, 2H, **3**), 3.33 – 3.03 (m, 2H, **19**), 2.86 (t, *J*=2.5, 1H, **16**), [2.43 – 2.31 (m) **9 minor**; 2.31 – 2.15 (m) **9 major,** 1H], [1.96 (s) **18 minor**; 1.95 (s) **18 major**, 3H], 1.55 – 1.40 (m, 3H, **20**), 1.41 – 1.26 (m, 3H, **21**), [1.02 (d, *J*=6.6) **14 minor**; 0.93 (d, *J*=6.6) **14 major**, 3H], [0.99 (d, *J*=6.6) **15 minor**; 0.91 – 0.84 (m) **15 major**, 3H], 0.91 – 0.84 (m, 3H, **22**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{16}H_{27}N_3O_3$ + H: 310.21; found 310.87.

fcba

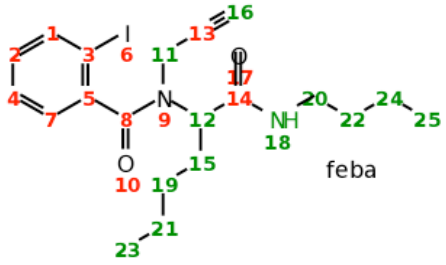


Yield: 173mg (81%, 0.41 mmol), yellow syrup

^{1}H NMR (400 MHz, Acetone-d_6) δ = [7.95 – 7.85 (m); 7.58 – 7.46 (m); 7.46 – 7.33 (m); 7.26 – 7.12 (m), 4H, **aromatic]**, 7.05 (s, **NH-18**), [4.75 – 4.71 (m); 4.68 (d, *J*=2.6); 4.65 (d, *J*=2.6); 4.63 – 4.57 (m); 4.57 – 4.52 (m); 4.34 (d, *J*=2.5); 4.30 (d, *J*=2.5), 2H, **11**], 3.98 – 3.79 (m, 1H, **12**), 3.32 – 3.07 (m, 2H, **20**), 2.63 (t, *J*=2.5, 1H, **16**), [2.17 – 2.06 (m); 2.02 – 1.97 (m); 1.96 – 1.87 (m), 2H, **15**], 1.58 – 1.41 (m, 2H, **21**), 1.41 – 1.24 (m, 2H, **22**), 1.11 – 1.00 (m, 3H, **19**), 0.98 – 0.84 (m, 3H, **23**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{18}H_{23}IN_2O_2$ + H: 427.08; found 427.29.

feba

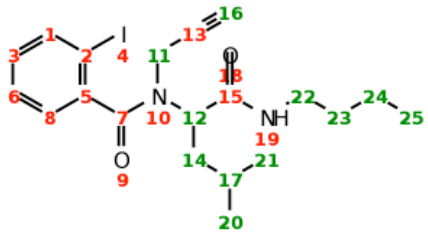


Yield: 191mg (84%, 0.42 mmol), yellow syrup

^{1}H NMR (400 MHz, Acetone-d_6) δ = [8.03 – 7.82 (m); 7.57 – 7.46 (m); 7.45 – 7.32 (m); 7.28 – 7.12 (m), 4H, **aromatic]**, 7.08 (s, NH-**18**), [4.75 – 4.72 (m); 4.71 – 4.67 (m); 4.66 (d, *J*=2.6); 4.64 – 4.57 (m); 4.57 – 4.54 (m); 4.31 (d, *J*=2.6); 4.27 (d, *J*=2.5), 2H, **11**], [4.02 – 3.91 (m); 3.94 – 3.86 (m) 1H, **12]**, 3.34 – 3.07 (m, 2H, **20**), 2.64 (t, *J*=2.5, 1H, **16**), 1.99 – 1.81 (m, 2H, **15**), 1.57 – 1.22 (m, 8H, **19, 21, 22, 24**), 1.00 – 0.81 (m, 6H, **23, 25**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{20}H_{27}IN_2O_2$ + H: 455.12; found 455.55.

ffba

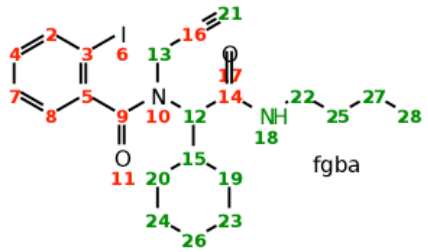


Yield: 140mg (63%, 0.31 mmol), yellow syrup

^{1}H NMR (400 MHz, Acetone-d_6) δ = [7.98 – 7.84 (m); 7.55 – 7.45 (m); 7.46 – 7.32 (m); 7.29 – 7.06 (m) 4H, **aromatic]**, [4.74 – 4.70 (m); 4.68 (d, *J*=2.3); 4.66 (d, *J*=2.7); 4.62 (d, *J*=2.5); 4.58 (d, *J*=2.5); 4.55 – 4.52 (m); 4.25 (d, *J*=2.6); 4.21 (d, *J*=2.6), 2H, **11**], 4.11 – 3.96 (m, 1H, **12**), 3.32 – 3.04 (m, 2H, **22**), 2.66 – 2.59 (m, 1H, **16**), 2.01 – 1.66 (m, 3H, **14, 17**), 1.62 – 1.41 (m, 2H, **23**), 1.44 – 1.23 (m, 2H, **24**), 1.10 – 0.71 (m, 9H, **20**, **21**, **25**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{20}H_{27}IN_2O_2$ + H: 455.12; found 455.30.

fgba

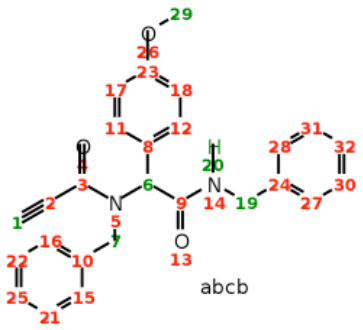


Yield: 180mg (75%, 0.37 mmol), white-yellow solid

^{1}H NMR (400 MHz, Acetone-d_6) δ = [7.95 – 7.82 (m); 7.55 – 7.44 (m); **7**.40 – 7.27 (m**);** 7.23 – 7.09 (m), 4H, **aromatic]**, 7.45 – 7.40 (m, **NH-18**), [5.07 (d, *J*=2.5); 5.03 (d, *J*=2.6); 4.91 – 4.74 (m); 4.59 (d, *J*=2.5); 4.56 (d, *J*=2.5); 4.55 (d, *J*=2.4); 4.52 (d, *J*=2.5), 2H, **13]**, [4.91 – 4.74 (m); 3.75 – 3.52 (m), 1H, **12**], 3.33 – 3.03 (m, 2H, **22**), 2.70 – 2.58 (m, 1H, **21**), 2.34 – 2.12 (m, 1H, **15**), 1.87 – 1.00 (m, 14H, **19**, **20**, **23**, **24**, **25**, **26**, **27**), 0.99 – 0.81 (m, 3H, **28**) ppm.

ESI-LRMS (*m/z*): calcd. for $C_{22}H_{29}IN_2O_2$ + H: 481.14; found 481.10

abcb



Yield: 151mg (73%, 0.37 mmol), yellow syrup
R_f (20:1 DCM/isopropylalcohol): 0.57

^{1}H NMR (400 MHz, $CDCl_3$) δ = [7.90 – 7.78 (m); 7.42 – 6.96 (m); 6.74 – 6.60 (m), 14H, **aromatic]**, [6.17 – 6.13 (m); 6.02 (t, *J*=5.4), **NH-20**], [5.61 (s) **6 minor**; 5.56 (s) **6 major**, 1H**]**, [5.11 (d, *J*=16.6); 4.64 (d, *J*=16.5), 2H, **7**], [4.48 (d, *J*=5.9); 4.44 (d, *J*=5.9); 4.39 (d, *J*=5.6); 4.37 – 4.33 (m), 2H, **19]**, [3.89 (s) **29 minor**; 3.73 (s) **29 major**, 3H**]**, [3.22 (s) **1 minor**; 3.09 (s) **1 major**, 1H] ppm.

ESI-LRMS (*m/z*): calcd. for $C_{26}H_{24}N_2O_3$ + H: 413.9; found 413.33.

General 'click'-procedure for Ugi-N-glucosamine-triazoles: BI.a-m.a-g.a-h.a-b

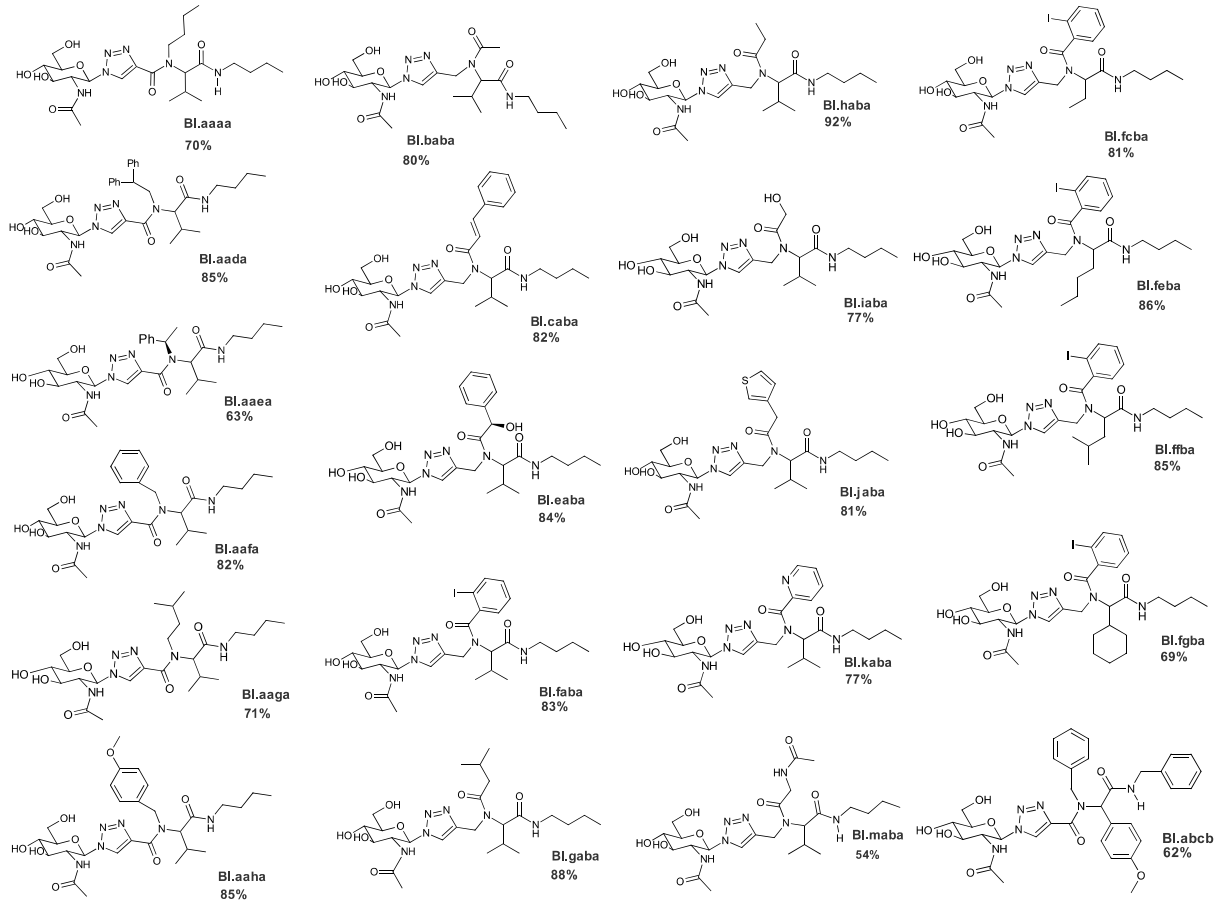


Figure S2 – Structure of GNTs and tested for anti-bacterial activity. Percentage values correspond to isolated yields for the glycosyl azide – alkyne coupling.

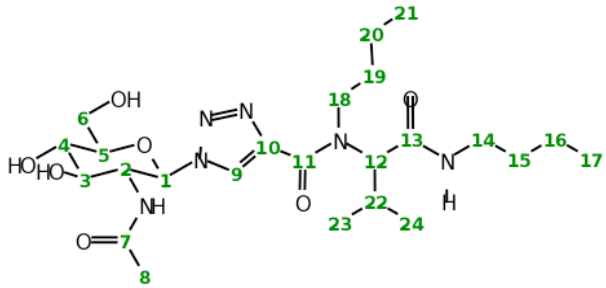
The reactions were run using stock solutions of the 2-*N*-Acetylamido-2-deoxy-β-D-glucopyranosyl azide (**1**)[1], the catalyst system and the solvent mixture.

For a 20 mg scale of (4) (0.081mmol):

The Ugi-compound (0.11mmol, 1.3 eq.) was dissolved in a 1:1 MeCN/H_2O solution (4.05 mL, 2.0mM). To this solution $CuSO_4 \cdot H_2O$ (10.00 µL of 0.8M $CuSO_4 \cdot H_2O$ in H_2O, 10mol%, 8.10µmol) and Cu-powder (10.00 µL of a 5.7M Cu-powder solution in H_2O, 0.06mmol, 0.7 eq.) was added. Lastly **1** (71.00µL of a 1.1M solution in H_2O, 0.08mmol) was added and the reaction was stirred at 45°C in a capped scintillation vial in an aluminum block until TLC showed completion. For the TLC the following solvents could be applied: 5:1 DCM/MeOH, 3:1

[1] Hong, S. Y.; Tobias, G.; Ballesteros, B.; El Oualid, F.; Errey, J. C.; Doores, K. J.; Kirkland, A. I.; Nellist, P. D.; Green, M. L. H.; Davis, B. G. Atomic-Scale Detection of Organic Molecules Coupled to Single-Walled Carbon Nanotubes. *J. Am. Chem. Soc.* **2007**, *129*, 10966–7.

DCM/MeOH or 2:1 DCM/MeOH. For visualization of the TLC *p*-anisaldehyde stain was used. After completion of the reaction 3-4 spatulas of CupriSorb™ and H_2O (2-3.00mL) were added and the mixture was **slowly** stirred at room temperature overnight. The content was carefully transferred to a new scintillation vial, avoiding the CupriSorb™ beads. H_2O, MeOH or DCM was used for rinsing the CupriSorb™ beads and the reaction vial and the MeCN/H_2O solvent mixture was removed using a centrifan. The residue was dissolved in 20:1 DCM/MeOH (1-2 mL) and purified with a silica-plug, where after approximately two test tubes the solvent was switched to either 3:1 or 5:1 DCM/MeOH. The residue left in the vial was treated with 3:1 or 5:1 DCM/MeOH and put on the same silica-plug, to elute the product. Collected fractions were tested with ESI-MS and TLC for any residual alkyne (20:1 DCM/MeOH, potassium permanganate stain) or **1** (3:1 or 5:1 DMC/MeOH, *p*-anisaldehyde stain). Clean product fractions were collected, the solvent residue was removed in vacuo and the product was dried under high vacuum.



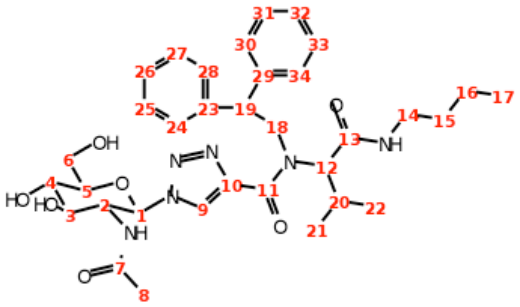
BI.aaaa

Yield: 17mg (78%, 0.03mmol), white solid
^{1}H NMR (600 MHz, MeOD) δ = [8.59 (s); 8.58 (s); 8.54 (s), 1H, **triazole-9]**, [5.85 (d, *J*=9.8); 5.83 (d, *J*=9.8); 5.79 (d, *J*=9.7); 5.77 (d, *J*=9.5), 1H, **1**], [4.56 (d, *J*=11.0); 4.51 (d, *J*=10.6); 4.36 (d, *J*=10.6), 1H, **12**], 4.27 – 4.05 (m, 1H, **2**), [3.96 – 3.88 (m); 3.83 – 3.73 (m), 2H, **6**], 3.74 – 3.65 (m, 1H, **3**), 3.66 – 3.54 (m, 2H, **4, 5**), [3.74 – 3.65 (m); 3.40 – 3.12 (m), 2H, **18**], 3.40 – 3.12 (m, 2H, **14**), [2.50 – 2.41 (m), **22 minor**; 2.41 – 2.33 (m), **22 major**, 1H], [1.80 (s); 1.79 (s); 1.78 (s), 3H, **8**], [1.74 – 1.61 (m); 1.60 – 1.42 (m), 2H, **19**], 1.60 – 1.42 (m, 2H, **15**), 1.43 – 1.27 (m, 2H, **16**), [1.43 – 1.27 (m); 1.25 – 1.16 (m), 2H, **20**], 1.06 – 0.72 (m, 12H, **23, 24, 21, 17**) ppm.

^{13}C NMR (151 MHz, MeOD) δ [173.3, 173.1, 171.7, 171.6, 164.6, 164.1- **7, 11, 13]**, [144.6, 144.4- **10]**, [128.2, 127.9, 126.9- **9]**, [88.9, 88.8, 88.3, 88.26- **1]**, [81.4, 81.3- **4, 5]**, [75.5, 75.3, 75.0- **3]**, [71.2, 71.15- **4, 5]**, [68.2, 67.9, 62.3, 62.2, 62.16- **6]**, [57.6, 57.3, 56.9, 56.87, 44.4, 44.1- **18]**, [40.1, 40.09, 40.0- **18, 14]**, [33.2, 33.1, 32.3, 32.2- **15]**, [30.8, 30.77- **19]**, [28.6, 28.4, 28.3, 28.2- **22]**, [22.6, 22.53, 22.52, 21.51, 21.4, 21.1, 21.08- **20, 16]**, [20.0, 19.7, 19.2, 19.1, 18.8- **23, 24]**, [14.2, 14.1, 14.05, 14.03- **17, 21]** ppm.

ESI(+)-HRMS (*m/z*): calcd. for $C_{24}H_{42}N_6O_7$ + H: 527.3193; found 527.3175.

BI.aada



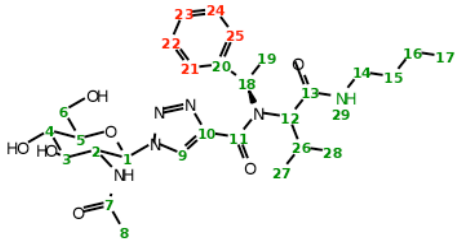
Yield: 34mg (85%, 0.05mmol), white solid

^{1}H NMR (600 MHz, MeOD) δ = [8.59 (s) **9 major**; 8.37 (s) **9 major**, 8.23 (s) **9 minor**; 8.17 (s) **9 minor**, 1H], 7.41 – 6.97 (m, 10H, **23-34**), 5.88 – 5.73 (m, 1H, **1**), 4.79 – 4.71 (m, 1H, **19**), [4.46 - 4.34 (m); 4.04 – 3.96 (m), 2H, **18**], [4.32 (d, *J*=10.7); 4.27 – 4.04 (m), 1H, **12**], 4.27 – 4.04 (m, 1H, **2**), [3.93 (dd, *J*=12.5, 2.1); 3.80 (dd, *J*=12.2, 5.1), 2H, **6**], 3.77 – 3.65 (m, 1H, **3**), 3.65 – 3.54 (m, 2H, **4, 5**), [3.23 – 3.01 (m); 2.84 – 2.58 (m), 2H, **14**], 2.36 (m, 1H, **20**), [1.80 (s); 1.79 (s); 1.75 (s), 3H, **8**], 1.45 – 1.23 (m, 4H, **15, 16**), 1.04 – 0.65 (m, 9H, **22**, **21**, **17**) ppm.

^{13}C NMR (151 MHz, MeOD) δ [173.3, 173.1, 172.9, 171.0, 170.98, 170.92, 166.0, 165.5- **7**, **11**, **13**], [144.7, 144.3, 144.0, 143.9, 143.8- **10**], [129.7, 129.6, 129.5, 129.4, 129.38, 129.31, 129.28, 129.22- **23-34**], [128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.56, 127.52, 127.4, 127.2- **9**], [88.8, 88.7- **1**], 81.4-81.2 (**4 or 5**), [75.6, 75.5, 75.2, 75.1- **3**], [71.3, 71.2- **4 or 5]**, [69.1, 68.9- **12**], [62.3, 62.2- **6**], [57.3, 57.2, 56.9- **2**], 51.3 (**18**), [48.8, 48.7, 48.5- **18, 19**], [40.2, 40.1- **14**], [32.14, 32.12, 32.0- **15**], [29.1, 28.5, 28.4- **20**], [22.6, 22.5- **8**], [21.15, 21.10- **16**], [20.0, 19.8, 19.7, 19.6, 19.4, 19.1, 18.8, 14.09, 14.06- **22, 21, 17**] ppm.

FAB-HRMS (*m/z*): calcd. for $C_{34}H_{46}N_6O_7$ + Na: 673.3326; found 673.3310.

BI.aaea

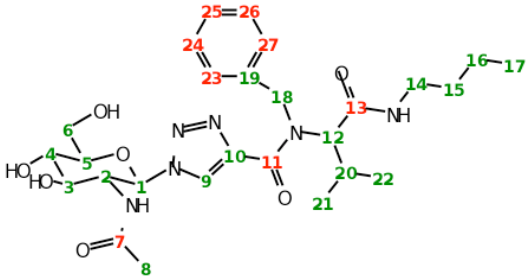


Yield: 15mg (63%, 0.03mmol), white solid
^{1}H NMR (600 MHz, MeOD) δ = [8.73 (s); 8.43 (s), 1H, **triazole-9**], 8.50 (s, **29-NH**), 7.53 – 7.05 (m, 5H, **21-25**), 5.92 – 5.70 (m, 1H, **1**), [5.92 – 5.70 (m); 5.47 – 5.22 (m), 1H, **18**], [5.47 – 5.22 (m); 4.47 (d, *J*=10.7), 1H, **12**], [4.32 – 4.22 (m); 4.12 – 4.04 (m), 1H, **2**], [3.97 – 3.86 (m); 3.80 (dd, *J*=12.3, 4.6), 2H, **6**], 3.75 – 3.67 (m, 1H, **3**), 3.66 – 3.56 (m, 2H, **4, 5**), [3.23 – 3.12 (m); 2.91 – 2.74 (m), 2H, **14**], 2.50 – 2.36 (m, 1H, **26**), [1.93 (d, *J*=7.1); 1.86 – 1.71 (m), 3H, **19**], 1.86 – 1.71 (m, 3H, **8**) 1.52 – 1.20 (m, 4H, **15, 16**), 1.19 – 0.71 (m, 9H, **17, 27, 28**) ppm.

^{13}C NMR (151 MHz, MeOD) δ [173.7, 173.4, 173.3, 171.4, 171.35, 165.6, 164.5- **7, 11, 13**], [145.0, 144.96, 144.92, 143.0, 140.0- **10**], [129.6, 129.5, 129.3, 129.2- **20**], [128.8, 128.4, 127.6, 127.3- **21-25**], [127.6, 126.6- **9**], [88.8, 88.51- **1**], [81.4, 81.3- **4 or 5**], [75.5, 74.9- **3**], [71.3, 71.2- **4 or 5**], [69.9, 69.5, 69.49, 68.8- **12**], [62.3, 62.1- **6**], 59.5 (**18**), [57.6, 56.9- **2**], 55.6 (**18**), [40.3, 40.2, 40.17, 40.0, 39.9, 39.86- **14**], [32.2, 32.1, 31.9- **15**], 29.2 (**26**), 22.5 (**8**), [21.2, 21.19, 21.15, 21.0- **16**], [20.1, 20.0, 19.7- **27, 28**], [18.7, 17.1- **19**], 14.1 (**17**) ppm.

ESI(+)-HRMS (*m/z*): calcd. for $C_{28}H_{42}N_6O_7$ + H: 575.3193; found 575.3191.

BI.aafa



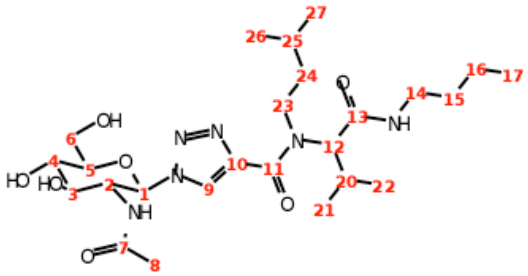
Yield: 37mg (82%, 0.07mmol), white solid
^{1}H NMR (600 MHz, MeOD) δ = [8.64 (s) **9 minor**; 8.61 (s) **9 major**; 8.49 (s) **9 minor**, 1H], 7.34 – 7.06 (m, 5H, **23-27**), 5.83 – 5.74 (m, 1H, **1**), [5.09 – 4.92 (m); 4.81 – 4.65 (m); 4.62 – 4.50 (m), 2H, **18**], [4.81 – 4.65 (m); 4.62 – 4.50 (m), 1H, **12**],4.23 – 4.07 (m, 1H, **2**), [3.96 – 3.85 (m); 3.85 – 3.77 (m), 2H, **6**], 3.77 – 3.64 (m, 1H, **3**), 3.65 – 3.51 (m, 2H, **4**, **5**), 3.20 – 2.84 (m, 2H, **14**), 2.53 – 2.39 (m, 1H, **20**), [1.80 (s); 1.79 (s); 1.69 (s); 1.65 (s), 3H, **8**], 1.50 – 1.25 (m, 4H, **15, 16**), 1.02 – 0.71 (m, 9H, **17, 21, 22**) ppm.

^{13}C NMR (151 MHz, MeOD) δ [173.3, 173.1, 171.4, 171.3, 165.2, 164.7- **13, 11, 7**], [144.5, 144.2- **10**], [139.2, 138.9- **19**], [129.19, 129.10, 129.07, 128.57, 128.52, 128.3, 127.9, 127.8, 127.2- **9, 23-37**], [88.84, 88.82- **1**], [81.37, 81.31- **4 or 5**], [75.3, 75.0- **3**], [71.29, 71.20- **4 or 5**], [68.8, 68.6- **12**], [62.23, 62.17- **6**], [57.6, 57.3- **2**], [47.4, 47.2- **18**], [40.1, 40.0- **14**], [32.23,

32.16- **15**], [29.0, 28.5- **20**], [22.6, 22.5- **8**], [21.13, 21.11- **16**], [20.0, 19.9, 19.7, 19.3, 19.1, 18.8, 14.1- **22, 21, 17**] ppm.

FAB-HRMS (*m/z*): calcd. for $C_{27}H_{40}N_6O_7$ + Na: 583.2856; found 583.2815.

BI.aaga

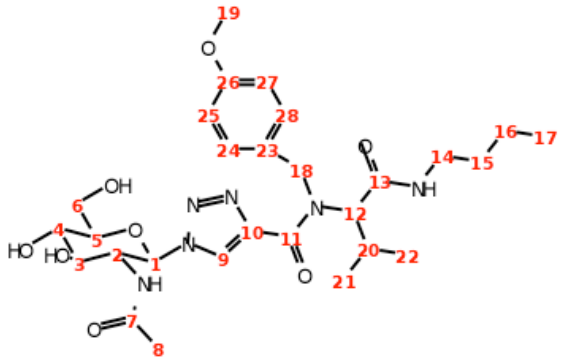


Yield: 31mg (71%, 0.06mmol), white solid
^{1}H NMR (600 MHz, MeOD) δ = [8.58 (s) **9 major**; 8.52 (s) **9 major**; 8.43 (s) **9 minor**; 8.35 (s) **9 minor**, 1H], 5.89 – 5.74 (m, 1H, **1**), [4.57 – 4.46 (m) **12 major**; (d, *J*=10.6) **12 minor**, 1H], 4.27 – 4.05 (m, 1H, **2**), [3.97 – 3.87 (m); 3.85 – 3.74 (m), 2H, **6**], [3.85 – 3.74 (m); 3.42 – 3.14 (m), 2H, **23**], 3.74 – 3.66 (m, 1H, **3**), 3.65 – 3.55 (m, 2H, **4, 5**), 3.42 – 3.14 (m, 2H, **14**), [2.51 – 2.42 (m) **20 minor**; 2.42 – 2.32 (m) **20 major**, 1H], [1.80 (s); 1.79 (s); 1.78 (s) 3H, **8**], 1.66 – 1.29 (m, 7H, **24**, **25**, **15**, **16**), 1.06 – 0.70 (m, 15H, **17, 21, 22, 26, 27**) ppm.

^{13}C NMR (151 MHz, MeOD) δ [173.3, 173.1, 171.7, 171.6, 171.5, 164.5, 163.9- **7, 11, 13**], [144.7, 144.5- **10**], [127.8, 126.9- **9**], [88.89, 88.82- **1**], [81.39, 81.34- **4 or 5**], [75.3, 75.0- **3**], 71.22 (**4 or 5**), [68.2, 67.9- **12**], [62.24, 62.20- **6**], [57.6, 57.3- **2**], [43.7, 42.9- **23**], [40.1, 39.9- **14**], 37.4 (**24**), [32.3, 32.2- **15**], [28.6, 28.0, 27.9- **20, 25**], [22.9, 22.88, 22.85, 22.61, 22.52- **8, 17, 21, 22, 26, 27**], [21.08, 21.06- **16**], [19.7, 19.1, 18.8, 14.04, 14.02- **17, 21, 22, 26, 27**] ppm.

FAB-HRMS (*m/z*): calcd. for $C_{25}H_{44}N_6O_7$ + Na: 563.3169; found 563.3152.

BI.aaha



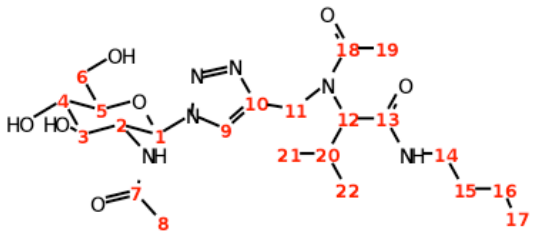
Yield: 41mg (85%, 0.07mmol), white solid
^{1}H NMR (600 MHz, MeOD) δ = [8.63 (s); 8.59 (s); 8.49 (s), 1H, **9**], [7.30 – 7.21 (m); 7.09 – 6.99; 6.85 – 6.72 (m), 4H, **24, 25, 27, 28**], 5.84 – 5.76 (m, 1H, **1**), [5.04 – 4.89 (m); 4.67 (d,

J=14.8); 4.47 (d, *J*=14.8), 2H, **18**], [4.64 (d, *J*=10.6); 4.51 (d, *J*=10.6), 1H, **12**], 4.24 – 4.08 (m, 1H, **2**), [3.96 – 3.85 (m); 3.83 – 3.77 (m), 2H, **6]**, 3.78 – 3.66 (m, 4H, **3, 19**), 3.65 – 3.52 (m, 2H, **4**, **5**), 3.21 – 2.97 (m, 2H, **14**), 2.57 – 2.39 (m, 1H, **20**), [1.80 (s) **8 major**; 1.78 (s) **8 major**; 1.71 (s) **8 minor**; 1.67 (s) **8 minor**, 3H], 1.49 – 1.35 (m, 2H, **15**), 1.37 – 1.24 (m, 2H, **16**), 1.03 – 0.73 (m, 9H, **17, 21, 22**) ppm.

^{13}C NMR (151 MH0z, MeOD) δ [173.3, 173.1, 171.5, 171.4, 171.37, 171.3, 165.1, 164.7, 160.16, 160.09- **7, 11, 13**], [144.5, 144.3- **10**], [131.2, 130.9- **23, 26**], [130.3, 130.27, 129.8, 129.6- **24, 25, 27, 28**], [128.2, 127.3- **9**], [114.7, 114.4- **24, 25, 27, 28**], 88.8 (**1**), [81.36, 81.31- **4 or 5**], [75.3, 75.0- **3**], [71.3, 71.2- **4 or 5**], [68.75, 68.71, 68.53, 68.5- **12**], [62.3, 62.22, 62.17- **6]**, [57.6, 57.3- **2**], 55.6 (**19**), [46.8, 46.6- **18**], [40.2, 40.0- **14**], [32.2, 32.17, 32.15- **15**], [28.9, 28.5- **20]**, [22.6, 22.5- **8**], [21.12, 21.10- **16**], [19.8, 19.1, 18.8, 14.1, 14.05- **17, 21, 22**] ppm.

FAB-HRMS (*m/z*): calcd. for $C_{28}H_{42}N_6O_8$ + Na: 613.2962; found 613.2950.

BI.baba



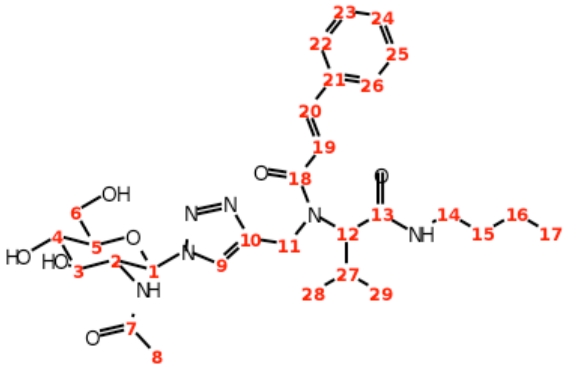
Yield: 32mg (80%, 0.07 mmol), white solid

^{1}H NMR (600 MHz, MeOD) δ = [7.98 (s); 7.92 (s), 1H, **9]**, 5.81 – 5.73 (m, 1H, **1**), [4.85 – 4.79 (m); 4.79 – 4.65 (m), 2H, **11]**, [4.61 (d, *J*=10.8); 4.56 (d, *J*=10.8); 3.85 – 3.80 (m), 1H, **12**], 4.24 – 4.11 (m, 1H, **2**), [3.96 – 3.85 (m); 3.80 – 3.63 (m), 2H, **6]**, 3.80 – 3.63 (m, 1H, **3**), 3.63 – 3.48 (m, 2H, **4, 5**), [3.27 – 3.08; 3.08 – 2.93 (m, 2H, **14]**, 2.54 – 2.28 (m, 1H-**20**), [2.27 (s); 2.21 (s); 2.19 (s); 2.16 (s) 3H, **19]**, [1.77 (s) **8 major**; 1.75 (s) **8 minor**, 3H], 1.57 – 1.44 (m, 2H, **15**), 1.44 – 1.26 (m, 2H, **16**), 1.06 – 0.61 (m, 9H, **17, 21, 22**) ppm.

^{13}C NMR (151 MHz, MeOD) δ 175.3, 175.1 (**7/13/18**), 173.4, 173.36 (**7/13/18**), 172.2, 172.1, 172.06 (**7/13/18**), 145.8 (**10**), 123.2, 122.8 (**9**), 88.30, 88.25, 88.0 (**1**), 81.46, 81.40, 81.3 (**5 or 4**), 76.1, 75.81, 75.7 (**3**), 71.6, 71.4 (**5 or 4**), 69.49, 69.44, 69.38, 69.32 (**12**), 64.96, 64.92 (**12**), 62.56, 62.54, 62.4 (**6**), 57.1, 56.9, 56.5 (**2**), 42.4, 42.02 (**11**), 40.38, 40.34, 40.31, 40.23, 40.21, 40.18 (**14**), 39.6 (**11**), 32.4, 32.38, 32.32 (**15**), 29.6, 28.6, 28.5 (**20**), 22.8, 22.7, 22.55, 22.50, 22.4 (**19/8**), 21.28, 21.26, 21.21 (**16**), 20.2, 20.1 (**22/21/17**), 19.8, 19.79, 19.74 **(22/21/17**), 19.37, 19.31 (**22/ 21/17**), 14.24, 14.21, 14.1 (**22/21/ 17**) ppm.

ES(+)I-HRMS (*m/z*): calcd. for $C_{22}H_{38}N_6O_7$ + H: 499.2880; found 499.2873.

BI.caba

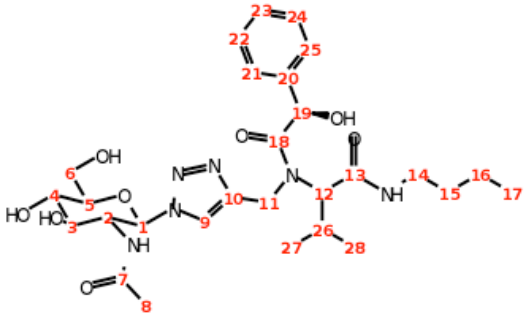


Yield: 39mg (82%, 0.07mmol), white solid
^{1}H NMR (600 MHz, MeOD) δ = [7.99 (s) **9 major**; 7.90 (s) **9 minor**, 1H], [7.71 – 7.53 (m); 7.46 – 7.32 (m); 7.28 – 7.13 (m), 7H, **19, 20, 22-26**], 5.83 – 5.73 (m, 1H, **1**), 5.10 – 4.79 (m, 2H, **11**), [4.74 (d, *J*=10.8); 4.70 (d, *J*=10.8); 4.10 (d, *J*=10.5), 1H, **12**], 4.25 – 4.12 (m, 1H, **2**), [3.93 – 3.83 (m); 3.78 – 3.62 (m), 2H, **6**], 3.78 – 3.62 (m, 1H, **3**), 3.62 – 3.46 (m, 2H, **4, 5**), 3.30 – 3.01 (m, 2H, **14**), [2.61 – 2.52 (m) **27 minor**; 2.52 – 2.45 (m) **27 minor**; 2.44 – 2.31 (m) **27 major**, 1H], [1.77 (s) **8 minor**; 1.73 (s) **8 minor**; 1.70 (s) **8 major**; 1.59 (s) **8 major**, 3H], 1.57 – 1.41 (m, 2H, **15**), 1.41 – 1.26 (m, 2H, **16**), 0.97 – 0.86 (m, 3H, **17**), [1.02 (d, *J*=6.4), **28 minor**; 0.99 (d, *J*=6.4), **28 major**, 3H], 0.97 – 0.86 (m, 3H, **17**), [0.86 – 0.78 (m) **29 major**; 0.73 (d, *J*=6.6) **29 minor**; 0.70 (d, *J*=6.6) **29 minor**, 3H] ppm.

^{13}C NMR (151 MHz, MeOD) δ [173.3, 173.1, 172.12 172.1, 171.8, 171.70, 170.28, 170.05- **7, 13, 18**], [146.8, 146.2- **10**], [144.9, 144.8- **20**], 136.4 (**21**), [131.0, 129.94, 129.91, 129.3, 129.2- **19, 21-26**], [123.3, 123.0- **9**], [119.7, 119.4, 119.2- **19, 21-26**], 88.1 (**1**), 81.3 (**4 or 5**), [75.7, 75.6- **3**], 71.5 (**4 or 5**), [68.9, 68.7, 65.0, 64.9- **12**], 62.4 (**6**), [57.0, 56.59- **2**], [41.4, 41.1- **11**], [40.2, 40.1- **14**], [32.3, 32.25- **15**], [30.1, 30.0, 28.7, 28.6- **27**], [22.6, 22.57, 22.52- **8**], [21.13, 21.09- **16**], [20.2, 20.0, 19.99, 19.7, 19.4, 19.3- **28, 29**], [14.13, 14.10, 14.0- **17**] ppm.

FAB-HRMS (*m/z*): calcd. for $C_{29}H_{42}N_6O_7$ + Na: 609.3013; found 609.3030.

BI.eaba



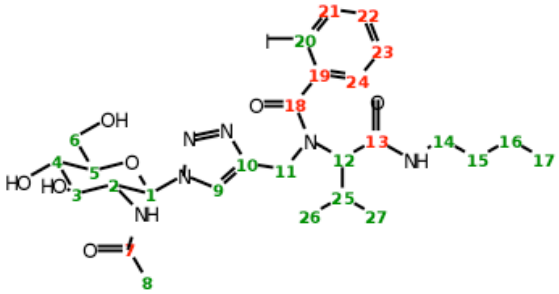
Yield: 40mg (84%, 0.07mmol), white solid
^{1}H NMR (600 MHz, MeOD) δ = [8.03 (s); 8.01(s); 7.97 (s); 7.96 (s); 7.92 (s), 1H, **9**], [7.90 – 7.75 (m); 7.58 – 7.27 (m), 5H, **21-25**], 5.84 – 5.54 (m, 2H, **1 and 19**), [5.07 – 4.72 (m); 4.69 –

4.62 (m); 4.57 – 4.48 (m), 2H, **11**], [4.62 – 4.57 (m); 4.48 – 4.43 (m); 4.02 – 3.93 (m), 1H, **12**], 4.28 – 4.10 (m, 1H, **2**), [3.94 – 3.84 (m); 3.84 – 3.65 (m), 2H, **6**], 3.84 – 3.65 (m, 1H, **3**), 3.65 – 3.49 (m, 2H, **4, 5**), [3.20 – 3.02 (m); 2.97 – 2.89 (m), 2H, **14**], 2.42 – 2.20 (m, 1H, **26**), [1.81 (s); 1.80 (s); 1.79 (s); 1.77 (s), 3H, **8**], 1.53 – 1.34 (m, 2H, **15**), 1.34 – 1.16 (m, 2H, **16**), 1.03 – 0.85 (m, 3H, **17**), [1.03 – 0.85 (m); 0.84 – 0.73 (m); 0.64 (d, *J*=6.6); 0.54 (d, *J*=6.7); 0.24 – 0.16 (m), 0.11 (d, *J*=6.7), 6H, **27, 28**] ppm.

^{13}C NMR (151 MHz, MeOD) δ [176.1, 175.7, 175.5, 174.8, 174.7, 173.5, 171.8, 171.4, 171.38- **7, 13, 18**], [145.3, 145.2- 2**0**], [140.5, 140.3, 140.1, 139.9- **10**], [129.99, 129.97, 129.90, 129.86, 129.7, 129.2, 128.84, 128.83, 127.5, 123.8- **21-25**], [123.3, 123.26- **9**], [88.2, 88.1, 87.9, 87.8- **1**], [81.24, 81.20- **4 or 5**], [75.9, 75.6, 75.5- **3**], [73.6, 73.4, 73.35- **19**], [71.5, 71.47, 71.3- **4 or 5**], [67.1, 66.7, 66.3, 64.6- **12**], [62.4, 62.3- **6**], [57.2, 57.0, 56.9, 56.4- **2**], 41.2 (**11**), [40.2, 40.1, 39.99, 39.90, 39.8- **11, 14**], [32.4, 32.2, 32.16, 32.1- **15**], [29.0, 28.8, 28.34, 28.30- **26**], [22.69, 22.66, 22.64- **8**], [21.1, 21.08, 21.05, 21.02- **16**], [20.2, 19.9, 19.7, 19.64, 19.63, 19.6, 18.8, 18.6, 18.58- **27, 28**], [14.12, 14.11, 14.04- **17**] ppm.

FAB-HRMS (*m/z*): calcd. for $C_{28}H_{42}N_6O_8$ + Na: 613.2962; found 613.2978.

BI.faba



Yield: 46mg (83%, 0.07mmol), white solid

^{1}H NMR (600 MHz, MeOD) δ = [8.27 (s); 8.19 (s); 8.17 (s); 8.16 (s); 7.64 (s), 1H, **9**], 7.96 – 7.85 (m); 7.54 – 7.03 (m), 4H, **21-24**], 5.94 – 5.65 (m, 1H, **1**), [5.44 (d, *J*=15.3); 5.37 (d, *J*=15.1); 5.27 – 5.16 (m); 5.12 – 4.91 (m); 4.73 – 4.38 (m), 2H, **11**], [4.73 – 4.38 (m); 3.82 – 3.38 (m), 1H, **12]**, 4.37 – 3.98 (m, 1H, **2**), [3.98 – 3.84 (m); 3.82 – 3.38 (m), 2H, **6**], 3.82 – 3.38 (m, 3H, **3, 4, 5**), 3.30 – 3.03 (m, 2H, **14**), 2.60 – 2.35 (m, 1H, **25**), [1.79 (s); 1.78 (s); 1.77 (s); 1.76 (s); 1.75 (s); 1.74 (s), 3H, **8**], 1.59 – 1.45 (m, 2H, **15**), 1.47 – 1.24 (m, 2H, **16**), 1.00 – 0.89 (m, 3H, **17**), [1.01 (d, *J*=6.5); 1.00 – 0.89 (m); 0.89 – 0.77 (m); 0.74 (d, *J*=6.4); 0.68 (d, *J*=6.4); 0.56 (d, *J*=6.5), 6 H, **26**, **27**] ppm.

^{13}C NMR (151 MHz, MeOD) δ [174.1, 174.0, 173.8, 173.4, 173.3, 173.1, 172.9, 172.3, 172.25, 171.8, 171.7- **7, 13, 18**], [143.15, 143.14, 143.0- **10**], [141.3, 141.1, 140.9, 140.84, 140.7, 140.4, 132.1, 132.09, 131.9, 131.7, 131.69, 129.9, 129.5, 129.49, 129.4, 129.2, 129.1, 128.7, 128.5- **19, 21-24**], [126.2, 126.1, 125.4, 124.9, 123.74, 123.72, 123.7- **9**], [93.8, 93.75, 93.5- **20**], [88.2, 88.01, 87.9, 87.86, 87.5- **1**], [81.4, 81.3, 81.29, 81.23, 81.2- **4 or 5]**, [76.8, 76.2, 75.8, 75.7, 75.65- **3]**, [71.6, 71.5, 71.4- **4 or 5**], [70.6, 70.5, 69.2, 65.8- **12**], [62.4, 62.2- **6**], [57.2, 57.1, 56.7, 56.68, 56.6, 56.5, 56.4- **2**], 43.6 (**11**), [40.2, 40.1, 39.98, 39.95- **11, 14**], [32.5, 32.4, 32.3, 32.1, 32.0- **15**], [30.4, 30.2, 30.0, 29.8, 29.0, 28.9, 28.84, 28.82- **25**], [22.89, 22.86, 22.7, 22.66- **8**], 21.4 (**16**), [21.3, 21.2, 21.08, 21.06, 20.8, 20.77, 20.4, 20.35, 20.30, 19.8, 19.7, 19.5, 19.45- **26, 27**], [14.1, 14.08, 14.05- **17**] ppm.

FAB-HRMS (*m/z*): calcd. for $C_{27}H_{39}IN_6O_7$ + Na: 709.1823; found 709.1846.

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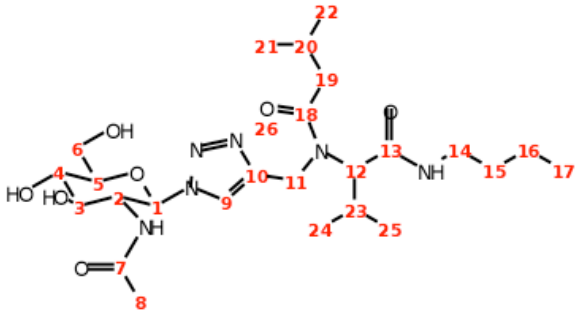
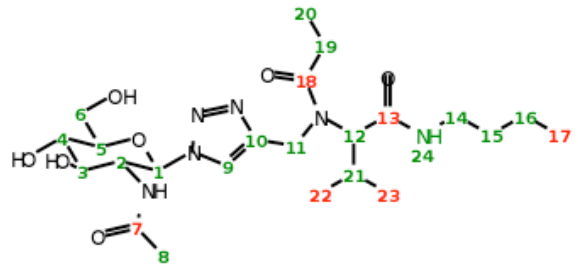


Yield: 39mg (88%, 0.07mmol), white solid
^{1}H NMR (600 MHz, MeOD) δ = [7.97 (s) **9 major**; 7.95 (s) **9 minor**; 7.89 (s) **9 minor**; 7.88 (s) **9 major**, 1H], 5.81 – 5.72 (m, 1H, **1**), [5.04 – 4.94 (m); 4.87 – 4.68 (m), 2H, **11**], [4.59 (d, *J*=10.8); 4.54 (d, *J*=10.8); 3.94 – 3.85 (m), 1H, **12**], 4.23 – 4.12 (m, 1H, **2**), [3.94 – 3.85 (m); 3.78 – 3.63 (m), 2H, **6**], 3.78 – 3.63 (m, 1H, **3**), 3.61 – 3.48 (m, 2H, **4, 5**), 3.25 – 2.97 (m, 2H, **14**), 2.56 – 2.08 (m, 4H, **19, 20, 23**), [1.78 (s) **8 major**; 1.76 (s) **8 minor**; 1.75 (s) **8 minor**, 3H], 1.56 – 1.38 (m, 2H, **15**), 1.41 – 1.26 (m, 2H, **16**), 1.05 – 0.88 (m, 3H, **17**), [1.05 – 0.88 (m); 0.83 – 0.75 (m); 0.69 – 0.60 (m), 12H, **21, 22, 24, 25**] ppm.

^{13}C NMR (151 MHz, MeOD) δ [176.7, 176.5, 175.4, 175.36, 173.3, 173.25, 173.1, 173.08, 172.3, 172.7, 172.1, 172.0, 171.9- **7, 13, 18**], [146.4, 145.8- **10**], [124.7, 123.9, 123.1, 122.7- **9**], [88.1, 88.06, 87.8, 87.7- **1**], [81.3, 81.27, 81.24, 81.22- **4 or 5**], [76.0, 75.7, 75.6- **3**], [71.5, 71.4, 71.3- **4 or 5**], [68.3, 68.2, 64.9- **12**], [62.5, 62.4, 62.38, 62.3- **6**], [57.1, 57.0, 56.7, 56.5- **2**], [43.7, 43.6, 43.1, 42.9- **19**], [41.8, 41.5- **11**], [40.2, 40.15, 40.1, 40.0, 39.9- **14**], [32.3, 32.28, 32.24, 32.22- **15**], [29.7, 29.6, 28.5, 28.3- **23**], [27.04, 27.01, 26.7, 26.6- **20**], [23.2, 23.1, 23.01, 22.99, 22.93, 22.90, 22.89, 22.87, 22.83, 22.7, 22.67, 22.63- **8, 21, 22, 24, 25**], [21.2, 21.1, 21.09- **16**], [20.2, 20.1, 20.0, 19.94, 19.91, 19.8, 19.7, 19.34, 19.30- **21, 22, 24, 25**], [14.12, 14.09, 14.07, 14.06- **17**] ppm.

FAB-HRMS (*m/z*): calcd. for $C_{25}H_{44}N_6O_7$ + Na: 563.3169; found 563.3161.

BI.haba



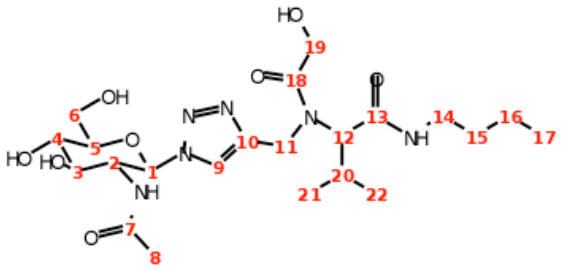
Yield: 38mg (92%, 0.07mmol), white solid
^{1}H NMR (600 MHz, MeOD) δ = [8.32 – 8.26 (m); 8.19 – 8.11 (m); 8.05 – 8.00 (m), **NH-24**],

[7.98 (s); 7.95 (s); 7.90 (s); 7.89 (s), 1H, **9**], 5.80 – 5.73 (m, 1H, **1**), [5.02 – 4.94 (m); 4.86 – 4.77(m); 4.75 – 4.67 (m), 2H, **11]**, [4.60 (d, *J*=10.7); 4.55 (d, *J*=10.8); 3.95 – 3.85 (m), 1H, **12]**, 4.26 – 4.11 (m, 1H, **2**), [3.95 – 3.85 (m); 3.80 – 3.61 (m), 2H, **6]**, 3.80 – 3.61 (m, 1H, **3**), 3.64 – 3.45 (m, 2H, **4, 5**), 3.27 – 2.96 (m, 2H, **14**), 2.69 – 2.27 (m, 3H, **19, 21**), [1.77 (s); 1.77 (s); 1.75 (s), 3H, **8]**, 1.56 – 1.26 (m, 4H, **15, 16**), 1.21 – 1.04 (m, 3H, **20**), 1.00 – 0.88 (m, 3H, **17**), [1.00 – 0.88 (m); 0.82 – 0.75; 0.66 (d, *J*=6.5), 0.63 (d, *J*=6.6), 6H, **22, 23]** ppm.

^{13}C NMR (151 MHz, MeOD) δ [178.1, 177.9, 176.8, 176.7, 173.38, 173.3, 173.2, 173.15, 172.2, 172.1, 171.96, 171.90- **7, 13, 18]**, [146.8, 146.4, 145.8- **10]**, [124.6, 123.8, 123.1, 122.6- **9]**,[88.1, 88.08, 87.83, 87.79- **1]**, [81.3, 81.24, 81.21- **4 or 5]**, [75.9, 75.6, 75.57- **3]**, [71.4, 71.35, 71.3- **4 or 5]**, [68.1, 67.9, 64.9- **12]**, [62.4, 62.3, 62.26- **6]**, [57.0, 56.9, 56.7, 56.3- **2]**, [41.6, 41.2- **11]**, [40.18, 40.1, 40.0- **14]**, 39.66 (**11**), [32.3, 32.2, 32.17- **15]**, [28.5, 28.4- **21]**, [28.1, 27.9, 27.8, 27.7- **19]**, [22.7, 22.6- **8]**, [21.2, 21.1, 21.08- **16]**, [20.2, 20.1, 19.8, 19.7, 19.68, 19.2, 19.15, 14.14, 14.11, 14.08- **7, 22, 23]**, [9.9, 9.84, 9.80- **20]** ppm.

FAB-HRMS (*m/z*): calcd. for $C_{23}H_{40}N_6O_7$ + H: 535.2856; found 535.2866.

BI.iaba



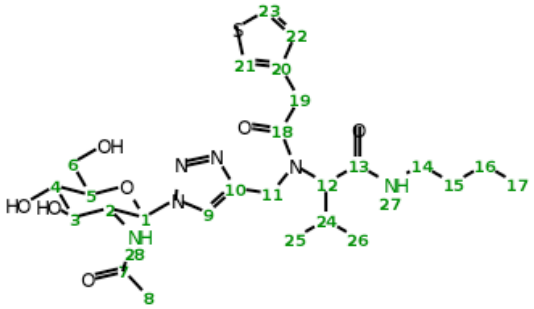
Yield: 32mg (77%, 0.06mmol), white solid

^{1}H NMR (600 MHz, MeOD) δ = [8.03 (s); 8.00 (s); 7.98 (s); 7.91 (s), 1H, **9**], 5.82 – 5.71 (m, 1H, **1**), [5.05 – 4.96 (m); 4.79 – 4.60 (m), 2H, **11**], [4.57 (d, *J*=10.9); 3.80 – 3.45 (m), 1H, **12**], [4.54 – 4.35 (m); 4.32 – 4.10 (m), 2H, **19**], 4.32 – 4.10 (m, 1H, **2**), [3.94 – 3.84 (m); 3.80 – 3.45 (m), 2H, **6]**, 3.80 – 3.45 (m, 3H, **4**, **5**, **3**), 3.29 – 2.91 (m, 2H, **14**), 2.53 – 2.28 (m, 1H, **20**), [1.78 (s); 1.77 (s); 1.75 (s), 3H, **8**], 1.55 – 1.25 (m, 4H, **15**, **16**), 1.00 – 0.88 (m, 3H, **17**), [1.00 – 0.88 (m); 0.82 – 0.75 (m); 0.66 (d, *J*=6.5); 0.61 (d, *J*=6.5), 6H, **22, 21**] ppm.

^{13}C NMR (151 MHz, MeOD) δ [175.61, 175.5, 174.4, 174.3, 173.5, 173.4, 171.8, 171.7, 171.4, 171.3, 164.9- **7**, **18**, **13**], [146.1, 145.9, 145.8, 145.3- 1**0**], [123.2, 122.9- **9**], [88.12, 88.10, 87.9- **1**], [81.27, 81.22, 81.2- **4 or 5**], [75.6, 75.58, 75.5- **3**], [71.5, 71.4, 71.35, 71.2- **4 or 5**], 66.8 (**12**), [65.2, 65.1, 62.4, 62.3, 62.2- **6**], [62.1, 61.9, 61.7- **19**], [56.9, 56.8, 56.2- **2**], [40.2, 40.1, 40.0- **14**], [39.6, 39.5, 39.4- **11**], [32.26, 32.25, 32.24, 32.2, 31.7- **14**], [29.3, 29.2, 28.5, 28.3- **20**], [22.7, 22.6, 22.56- **8**], [21.13, 21.11, 21.08- **16**], [19.98, 19.92, 19.7, 19.5, 19.49, 19.17, 19.16- **21, 22**], [14.14, 14.10, 14.07- **17**] ppm.

FAB-HRMS (*m/z*): calcd. for $C_{22}H_{38}N_6O_8$ + Na: 535.2649; found 535.2658.

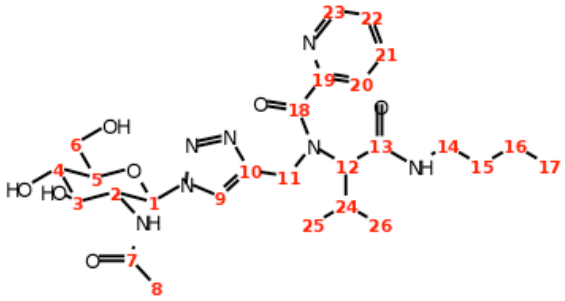
BI.jaba



Yield: 38mg (81%, 0.07mmol), white solid
^{1}H NMR (600 MHz, MeOD) δ = 8.10 (m, NH-**27, NH-28**), [8.00 (s); 7.97 (s); 7.96 (s); 7.95 (s), 1H, **9**], [8.54 (m); 7.92 (s); 7.46 – 7.34 (m); 7.29 – 7.18 (m); 7.10 – 7.01 (m), 3H, **21, 22, 23**], 5.83 – 5.74 (m, 1H, **1**), [4.99 – 4.80 (m); 4.79 – 4.66 (m), 2H, **11**], [4.62 – 4.53 (m); 3.96 – 3.80 (m), 1H, **12**], 4.27 – 4.12 (m, 1H, **2**), [4.08 – 4.02 (m); 3.96 – 3.80 (m), 2H, **19**], [3.96 – 3.80 (m); 3.80 – 3.65 (m), 2H, **6**], 3.80 – 3.65 (m, 1H, **3**), 3.65 – 3.49 (m, 2H, **4, 5**), 3.24 – 3.00 (m, 2H, **14**), 2.52 – 2.24 (m, 1H, **24**), [1.77 (s); 1.77 (s); 1.76 (s), 3H, **8**], 1.51 – 1.25 (m, 4H, **15, 16**), 0.98 – 0.88 (m, 3H, **17**), [0.98 – 0.88 (m); 0.75 – 0.67 (m); 0.48 (d, *J*=6.6), 0.46 (d, *J*=6.6), 6H, **26, 25**] ppm.

^{13}C NMR (151 MHz, MeOD) δ [175.07, 174.8, 173.3, 173.26, 173.1, 171.8- **7, 18, 13**], 149.9 (**21, 22, 23**), [146.3, 145.7- **10**], 138.5 (**21, 22, 23**), [135.9, 135.85, 135.8- **20**], [129.8, 129.5, 129.48, 127.2, 127.0, 126.8, 126.79, 123.9, 123.8, 123.7- **21, 22, 23**], [123.1, 122.8- **9**], [88.2, 88.16, 87.9, 87.7- **1**], [81.3, 81.2, 81.19, 81.16- **4 or 5**], [75.9, 75.6, 75.5- **3**], [71.4, 71.3, 71.2- **4 or 5**], [68.6, 68.4, 64.9 64.8- **12**], [62.4, 62.38, 62.32, 62.3- **6**], [56.9, 56.8- **2**], [41.6, 41.1- **11**], [40.2, 40.1, 40.02- **14**], [36.9, 36.88, 36.4, 36.39- **19**], [32.2, 32.18- **15**], [29.5, 28.6, 28.4- **24**], [22.7, 22.67, 22.65- **8**], [21.2, 21.1, 21.08- **16**], [19.7, 19.66, 19.3, 19.26, 19.17, 19.14- **26, 25, 17**], [14.16, 14.14, 14.1- **17**] ppm.
FAB-HRMS (*m/z*): calcd. for $C_{26}H_{40}N_6O_7S$ + Na: 603.2577; found 603.2561.

BI.kaba

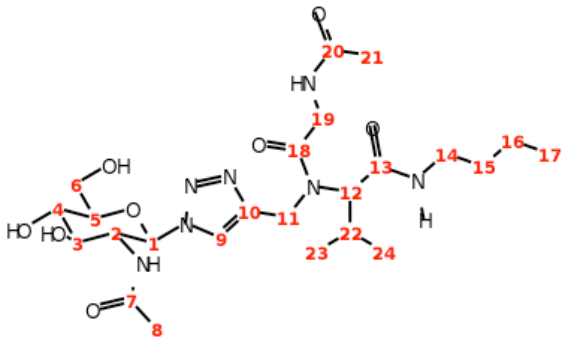


Yield: 32mg (77%, 0.06mmol), white solid
^{1}H NMR (600 MHz, MeOD) δ = [8.73 – 8.43 (m); 8.25 – 7.69 (m), 3H, **20-23**], [7.55 (s); 7.50 (s), 1H, **9**], 5.96 – 5.69 (m, 1H, **1**), [5.31 – 4.94 (m); 4.78 – 4.58 (m), 2H, **11**], [4.48 (m); 3.67 – 3.61 (m), 1H, **12**], 4.38 – 4.05 (m, 1H, **2**), [3.89 (m); 3.83 – 3.67 (m), 2H, **6**], 3.83 – 3.67 (m, 1H, **3**), 3.62 – 3.48 (m, 2H, **4, 5**), 3.30 – 3.04 (m, 2H, **14**), 2.58 – 2.41 (m, 1H, **24**), [1.83 (s); 1.77 (s); 1.73 (s), 3H, **8**], 1.60 – 1.44 (m, 2H, **15**), 1.44 – 1.24 (m, 2H, **16**), 1.09 – 0.54 (m, 9H, **26, 25, 17**) ppm.

13C NMR (151 MHz, MeOD) δ [173.3, 171.7, 171.49, 171.48- **7, 18, 13**], [151.5, 151.4, 151.2, 148.3, 148.2, 148.1- **20, 21, 22, 23**], [146.4, 146.3, 146.1- **10**], [136.7, 136.6, 136.5- **20, 21, 22, 23]**, [134.4, 134.2- **19**], [125.5, 124.7, 124.5, 124.4- **9**], 123.15 (**20, 21, 22, 23)**, [88.1, 87.9- **1**], 81.2 (**4 or 5**), [76.1, 76.0, 75.9, 75.8, 75.6- **3**], [71.43, 71.42- **4 or 5**], [70.3, 70.2, 70.1, 66.5, 66.4, 66.1- **12**], [62.4, 62.3- **6**], [56.88, 56.87, 56.5, 56.1- **2**], [44.0, 43.8- **11**], [40.3, 40.2, 40.1- **11, 14**], [32.3, 32.2- **15**], [29.9, 29.7, 28.8, 28.7, 28.6- **24**], [22.8, 22.7- **8**], [21.21, 21.17- **16**], [20.5, 20.4, 20.3, 20.2, 19.8, 19.6, 19.4- **26, 25**], 14.1 (**17**) ppm.

FAB-HRMS (*m/z*): calcd. for $C_{26}H_{39}N_7O_7$ + Na: 584.2809; found 584.2820.

BI.maba



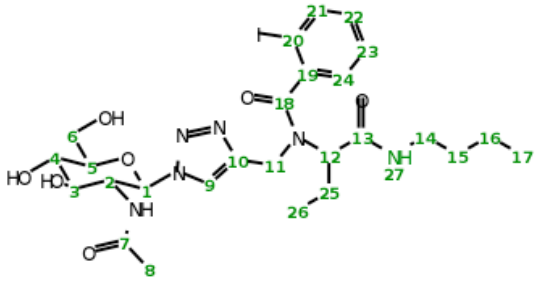
Yield: 25mg (54%, 0.04mmol), white solid

^{1}H NMR (600 MHz, MeOD) δ = [8.05 (s); 7.97 (s); 7.96 (s); 7.93 (s), 1H, **9**], 5.80 – 5.73 (m, 1H, **1**), 5.12 – 4.66 (m, 2H, **11**), [4.61 (d, *J*=10.9); 4.57 – 4.53 (m); 3.83 – 3.63 (m), 1H, **12**], 4.38 – 3.98 (m, 3H, **2, 19**), [3.95 – 3.85 (m); 3.83 – 3.63 (m), 2H, **6**], 3.83 – 3.63 (m, 1H, **3**) 3.63 – 3.48 (m, 2H, **4, 5**), 3.27 – 2.90 (m, 2H, **14**), 2.57 – 2.26 (m, 1H, **22**), [2.04 (s); 2.03 (s); 2.01 (s); 2.00 (s); 1.99 (s), 3H, **21**], [1.78 (s); 1.77 (s); 1.75 (s), 3H, **8**], 1.55 – 1.26 (m, 4H, **15, 16**), 1.04 – 0.85 (m, 3H, **17**), [1.04 – 0.85 (m); 0.84 – 0.76 (m); 0.71 (d, *J*=6.5), 0.65 (d, *J*=6.5), 6H, **23, 24**] ppm.

^{13}C NMR (151 MHz, MeOD) δ [173.4, 172.6, 172.5, 171.8, 171.7, 171.3, 171.1- **7, 13, 18, 20**], [146.1, 145.4- **10**], [123.3, 122.8- **9**], [88.1, 87.8- **1**], [81.23, 81.2- **4 or 5**], [75.8, 75.7, 75.6, 75.4- **3**], [71.5, 71.4, 71.3- **4 or 5**], [67.4, 67.37, 65.2, 65.15- **12**], [62.4, 62.38, 62.31- **6**], [57.1, 56.9, 56.5- **2**], [42.9, 42.7, 42.6, 42.5- **19**], [40.8, 40.5- **11**], [40.1, 40.09, 40.07- **14**], [39.7, 39.67- **11**], [32.2, 32.17- **15**], [29.6, 28.5, 28.4- **22**], [22.7, 22.6- **8**], [22.5, 22.4, 22.39- **21**], [21.1, 21.09- **16**], [20.0, 19.9, 19.6, 19.57, 19.55, 19.3, 19.2- **24, 25**], [14.1, 14.09, 14.07- **17**] ppm.

FAB-HRMS (*m/z*): calcd. for $C_{24}H_{41}N_7O_8$ + Na: 578.2914; found 578.2925.

BI.fcba

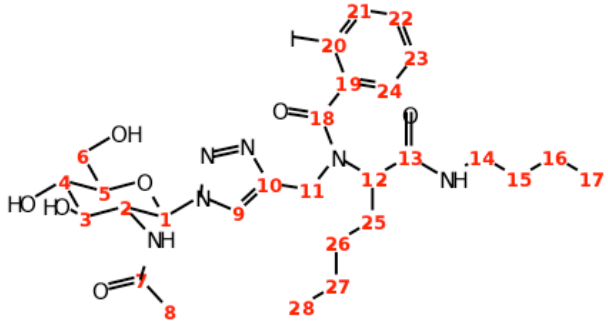


Yield: 42mg (77%, 0.06mmol), white solid
^{1}H NMR (600 MHz, MeOD) δ = [8.31 (s); 8.22 (s); 8.19 (s); 8.16 (s), 1H, **9**], [7.96 – 7.74 (m); 7.58 – 7.29 (m); 7.28 – 7.06 (m), 5H, **21-24, NH-27**], 5.94 – 5.70 (m, 1H, **1**), [5.22 – 5.03 (m); 4.84 – 4.56 (m); 4.50 – 4.40 (m), 2H, **11**], [4.40 – 4.04 (m); 4.01 – 3.85 (m); 3.84 – 3.46 (m), 1H, **12**], 4.40 – 4.04 (m, 1H, **2**), [4.01 – 3.85 (m); 3.84 – 3.46 (m), 2H, **6**], 3.84 – 3.46 (m, 3H, **4, 5, 3**], 3.27 – 3.03 (m, 2H, **14**), 2.21 – 1.84 (m, 2H, **25**), [1.81 (s); 1.79 (s); 1.76 (s); 1.63 (s); 1.62 (s), 3H, **8**], 1.60 – 1.40 (m, 2H, **15**), 1.40 – 1.26 (m, 2H, **16**), [1.10 (t, *J*=7.6); 1.07 – 1.00 (m); 0.77 (t, *J*=7.4); 0.74 – 0.70 (m); 0.68 (t, *J*=7.4); 0.57 (t, *J*=7.3), 3H, **26**], 1.00 – 0.85 (m, 3H, **17**) ppm.

^{13}C NMR (151 MHz, MeOD) δ [174.2, 174.1, 173.9, 173.2, 173.1, 172.9, 172.8, 172.6- **7, 13, 18**], [143.2, 143.1, 142.8, 142.7, 142.3- **10, 19**], [141.0, 140.9, 140.7, 140.65, 140.4, 132.0, 131.9, 131.86, 131.8, 129.7, 129.5, 129.4, 129.3, 129.28, 129.2, 128.7, 128.6, 128.0, 127.9- **21-24**], [126.1, 124.4, 124.2, 123.2, 123.1- **9**], [93.8, 93.76, 93.4, 93.1, 93.0, 92.9- **20**], [88.2, 88.1, 88.0, 87.9, 87.6- **1**], [81.4, 81.3, 81.2, 81.16- **4 or 5**], [76.6, 76.1, 75.7, 75.67, 75.5, 75.47- **3**], [71.6, 71.4, 71.3, 71.27- **4 or 5**], [66.4, 66.3, 66.2, 66.1, 64.6, 64.5, 62.9, 62.87- **12**], [62.6, 62.4, 62.3- **6**], [57.1, 56.9, 56.8, 56.7, 56.6, 56.4- **2**], [45.9, 45.5, 40.7- **11**], [40.4, 40.4, 40.3, 40.2, 40.0- **11, 14**], [32.5, 32.46, 32.4, 32.36, 32.3, 32.23, 32.21- **15**], [32.2, 26.3, 26.2, 25.7, 25.6- **25**], [23.3, 23.2, 22.9, 22.8, 22.76, 22.7- **8**], [21.3, 21.2, 21.1- **16**], [14.2, 14.1, 14.06- **17**], [12.8, 11.9, 11.87, 11.7, 11.5, 11.4- **26**] ppm.

FAB-HRMS (*m/z*): calcd. for $C_{26}H_{37}IN_6O_7$ + Na: 695.1666; found 695.1651.

BI.feba



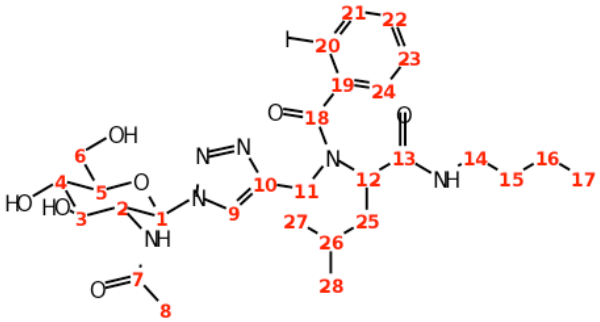
Yield: 49mg (86%, 0.07mmol), white solid
^{1}H NMR (600 MHz, MeOD) δ = [8.32 (s) **9 major**; 8.21 (s) **9 major**; 8.17 (s) **9 minor**; 8.16 (s) **9 minor,** 1H], [7.97 – 7.82 (m); 7.55 – 7.28 (m); 7.27 – 7.08 (m), 4H, **21-24**], 5.91 – 5.70 (m, 1H, **1**), [5.25 – 5.02 (m); 4.78 – 4.67 (m); 4.50 – 4.36 (m), 2H, **11**], 4.35 – 4.06 (m, 1H, **2**), [4.35

– 4.06 (m); 3.94 – 3.82 (m), 1H, **12**], [3.94 – 3.82 (m); 3.82 – 3.62 (m), 2H, **6**], 3.82 – 3.62 (m, 1H, **3**) 3.63 – 3.48 (m, 2H, **4, 5**), 3.30 – 3.03 (m, 2H, **14**), 2.24 – 1.84 (m, 2H, **25**), [1.82 (s); 1.81 (s); 1.80 (s); 1.79 (s); 1.75 (s), 3H, **8**], 1.64 – 0.99 (m, 8H, **26, 27, 15, 16**), 0.99 – 0.71 (m, 6H, **28, 17**) ppm.

^{13}C NMR (151 MHz, MeOD) δ [174.0, 173.9, 173.8, 173.5, 173.3, 173.2, 173.1, 172.9, 172.86, 172.80, 172.5, 172.49, 171.9- **7, 13, 18**], [146.2, 146.1, 145.7, 144.8, 144.7, 143.1, 143.06, 142.8, 142.6, 142.2- **10, 19**], [141.1, 140.9, 140.8, 140.7, 140.5, 131.9, 131.87, 129.6, 129.5, 129.3, 128.9, 128.7, 128.5, 128.1- **21, 22, 23, 24**], [125.6, 124.57, 124.5, 123.2- **9**], [93.9, 93.8, 93.3, 92.9- **20**], [88.2, 88.1, 87.9, 87.8- **1**], [81.3, 81.2, 81.17, 81.13, 81.1- **4 or 5**], [76.5, 75.9, 75.7, 75.66, 75.5, 75.48- **3**], [71.5, 71.4, 71.3- **4 or 5**], [64.9, 64.86, 64.8, 63.3- **12**], [62.5, 62.4, 62.3- **6**], [61.6, 61.3- **12**], [57.2, 56.9, 56.8, 56.7, 56.6, 56.5, 56.4- **2**], [46.0, 45.6, 45.5- **11**], [40.6, 40.4, 40.3, 40.2, 40.1, 39.9- **11, 14**], [32.8, 32.6, 32.4, 32.36, 32.3, 32.2, 32.19, 32.0, 31.9, 30.8, 30.7, 30.2, 30.1, 29.9, 29.8, 29.7, 29.69, 29.4- **15, 16, 25, 26, 27**], [23.8, 23.6, 23.5, 23.3, 23.2, 22.9, 22.9, 22.8, 22.7, 22.67- **8**], [21.3, 21.1- **15, 16, 25, 26, 27**], [14.4, 14.39, 14.3, 14.2, 14.16, 14.13, 14.1, 14.06- **28, 17**] ppm.

FAB-HRMS (*m/z*): calcd. for $C_{28}H_{41}IN_6O_7$ + Na: 723.1979; found 723.1952.

BI.ffba



Yield: 48mg (85%, 0.07mmol), white solid

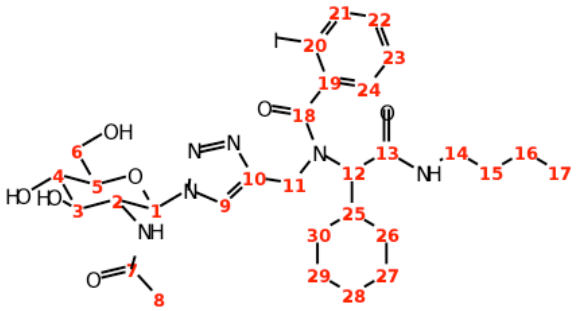
^{1}H NMR (600 MHz, MeOD) δ = [8.33 (s) **9 major**; 8.20 (s) **9 major**; 8.18 (s) **9 minor**; 8.14 (s) **9 minor**, 1H], [7.97 – 7.83 (m); 7.53 – 7.29 (m); 7.29 – 7.09 (m), 4H, **21-24]**, 5.92 – 5.69 (m, 1H, **1**), [5.23 – 4.99 (m); 4.78 – 4.53 (m); 4.49 – 4.38 (m), 2H, **11]**, [4.49 – 4.38 (m); 3.83 – 3.48 (m), 4.04 – 3.95 (m), 1H, **12]**, 4.35 – 4.04 (m, 1H, **2**), [3.94 – 3.86 (m); 3.83 – 3.48 (m), 2H, **6**], 3.83 – 3.48 (m, 3H, **4, 3, 5**), 3.30 – 3.09 (m, 2H, **14**), 2.06 – 1.91 (m, 2H, **25**), [1.86 (s); 1.82 (s); 1.81 (s); 1.80 (s); 1.75 (s), 3H, **8**], 1.66 – 1.26 (m, 5H, **26, 15, 16**), 1.14 – 0.56 (m, 9H, **28**, **27**, **17**) ppm.

^{13}C NMR (151 MHz, MeOD) δ [174.0, 173.9, 173.7, 173.4, 173.35, 173.1, 172.9, 172.8, 172.7, 172.4, 171.9- **7, 13, 18**], [146.2, 146.0, 145.8, 145.5, 144.7, 144.6, 143.0, 142.9, 142.7, 142.2, 141.8, 141.2, 140.9, 140.7, 140.5, 140.4- **10, 19**], [132.1, 131.9, 131.7, 129.6, 129.5, 129.2, 128.9, 128.7, 128.6, 127.9- **24, 23, 22, 21**], [126.0, 124.6, 124.5, 124.4, 123.5, 123.2- **9**], [94.5, 94.4, 93.4, 93.1, 93.0, 92.9- **20**], [88.2, 88.1, 87.9, 87.85, 87.7- **1**], [81.3, 81.2, 81.14, 81.10- **4 or 5**], [76.7, 76.0, 75.7, 75.67, 75.5, 75.46- **3**], [71.6, 71.4, 71.3- **4 or 5**], [69.8, 62.83, 62.8, 62.6, 62.4, 62.3- **6, 12**], [61.4, 61.3, 59.2, 58.9- **12**], [57.1, 56.9, 56.7, 56.5, 56.3- **2**], [45.5, 45.48, 45.3, 43.2, 41.9- **11**], [40.8, 40.7, 40.6, 40.5, 40.3, 40.28, 40.24, 40.15, 39.9- **11, 14, 25**], [38.9, 38.8-

25], [32.5, 32.4, 32.2- **15**], [26.24, 26.21, 25.7, 25.6, 25.4- **26**], [23.0, 22.93, 22.90, 22.86, 22.8, 22.75, 22.70, 22.6- **8, 28, 27, 17**], [21.3, 21.2, 21.1- **16**], [14.13, 14.09- **28, 27, 17**] ppm.

FAB-HRMS (*m/z*): calcd. for $C_{28}H_{41}IN_6O_7$ + Na: 723.1979; found 723.1954.

BI.fgba



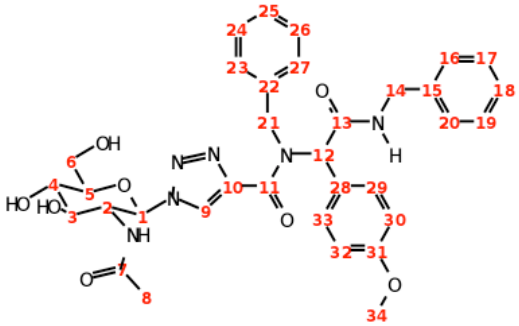
Yield: 41mg (69%, 0.06mmol), white solid

^{1}H NMR (600 MHz, MeOD) δ = [8.30 (s); 8.22 (s), 8.19 (s), 8.18 (s), 1H, **9**], [8.08 – 7.77 (m); 7.73 – 7.32 (m); 7.31 – 6.98 (m), 4H, **21-24**], 5.91 – 5.68 (m, 1H, **1**), [5.54 – 4.97 (m); 4.59 – 4.36 (m), 2H, **11**], [4.76 (d, *J*=11.4); 4.67 (d, *J*=11.8); 4.66 – 4.61 (m); 3.61 – 3.44 (m), 1H, **12**], 4.35 – 4.00 (m, 1H, **2**), [3.99 – 3.83 (m); 3.84 – 3.61 (m), 2H, **6**], 3.84 – 3.61 (m, 1H, **3**), 3.61 – 3.44 (m, 2H, **4, 5**), 3.28 – 3.02 (m, 2H, **14**), 2.52 – 1.90 (m, 1H, **25**), [1.83 (s); 1.80 (s); 1.78 (s); 1.76 (s), 3H, **8**], 1.75 – 0.54 (m, 17H, **30**, **29**, **28**, **27**, **26**, **17**, **16**, **15**) ppm.

^{13}C NMR (151 MHz, MeOD) δ [174.3, 173.4, 173.1, 172.9- **7, 13, 18**], [143.2, 143.2, 143.16- **10, 19**], [141.3, 141.1, 140.9, 140.8, 132.1, 131.9, 131.7, 129.6, 129.5, 129.4, 129.2, 129.1, 128.6, 128.5- **21-24**], [123.8, 123.7, 123.5- **9**], [94.2, 93.9, 93.7, 93.5, 93.0- **20**], [88.2, 88.1, 87.8- **1**], [81.4, 81.3, 81.2, 81.1, 81.0- **4 or 5**], [76.2, 75.8, 75.7, 75.6, 75.4- **3**], [71.6, 71.4, 71.36, 71.3- **4 or 5**], [69.7, 69.6, 68.24, 68.21, 64.4- **12**], [62.6, 62.44, 62.40- **6**], [57.3, 57.1, 56.7, 56.6, 56.4, 56.3- **2**], [40.2, 40.1, 39.95, 39.92- **11, 14**], [39.3, 39.2, 39.1, 38.8, 38.1, 37.9- **25**], [32.5, 32.3, 32.2, 32.1, 32.0- **15**], [31.6, 31.3, 30.8, 30.7, 30.6, 27.4, 27.3, 27.0, 26.9, 26.88, 26.6, 26.5, 26.3- **26-30**], [22.98, 22.91, 22.8, 22.7- **8**], [21.4, 21.31, 21.28, 21.1- **16**], [14.2, 14.10, 14.08- **17**] ppm.

FAB-HRMS (*m/z*): calcd. for $C_{30}H_{43}IN_6O_7$ + Na: 749.2136; found 749.2155.

BI.abcb



Yield: 33mg (62%, 0.05mmol), white solid

^{1}H NMR (600 MHz, MeOD) δ = [8.68 (s); 8.60 (s); 8.55 (s); 8.52 (s), 1H, **9**], 7.64 – 6.39 (m, 14H, **16-20, 23-27, 29, 30, 32, 33**), 5.98 – 5.76 (m, 1H, **1**), [6.60 – 6.38 (m); 5.98 – 5.76 (m), 1H, **12**], [5.98 – 5.76 (m); 5.66 – 5.49 (m); 5.25 – 5.01 (m); 4.80 – 4.47 (m), 2H, **14**], 4.49 – 4.29 (m, 2H, **21**), 4.25 – 4.04 (m, 1H, **2**), [3.98 – 3.85 (m); 3.85 – 3.45 (m), 2H, **6**], 3.85 – 3.45 (m, 6H, **4, 3, 5, 34**), [1.78 (s), 1.75 – 1.62 (m), 3H, **8**] ppm.

^{13}C NMR (151 MHz, MeOD) δ [173.7, 173.5, 173.4, 172.5, 172.4, 164.9, 164.9, 164.8, 164.7, 164.6, 161.4- **7, 11, 13**], [144.6, 144.5, 144.4- **10**], [139.8, 139.77, 139.5, 139.4- **15, 22, 28, 31**], [133.0, 132.9, 132.5, 132.4, 132.1, 132.06, 129.5, 129.45, 128.9, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 127.9, 127.8, 127.79, 127.5, 127.47, 127.2, 126.8, 114.9, 114.8, 114.7- **9, 16-20, 23-27, 29, 30, 32, 33**], [88.6, 88.5, 88.3, 88.2, 88.1- **1**], 81.4 (**4 or 5**), [75.5, 75.48, 75.4, 75.3- **3**], 71.3 (**4 or 5**), [65.1, 65.0- **12**], [62.31, 62.27- **6**], [57.4, 57.4, 56.9, 56.87- **2**], 55.7 (**34**), [51.9, 51.8, 51.6, 51.55, 51.5- **14**], 44.1 (**21**), [22.5, 22.47, 22.45- **8**] ppm.

ESI(+)-HRMS (*m/z*): calcd. for $C_{34}H_{38}N_6O_8$ + H: 659.2829; found 659.2813.

Anti-microbial testing

Bacterial strains and growth conditions. *Clostridium difficile* ATCC 70057 (tcdA-, tcdB-) was grown in supplemented brain heart infusion (BHIS) containing 0.1% cysteine and 0.5% yeast extract at 37 °C under anaerobic conditions (Whitley DG250 workstation). *Bacillus subtilis* ATCC 11775 and *Bacillus cereus* were cultured in either Nutrient Broth (NB) or LB at 30 °C. Streptococcus pneumoniae ATCC 6501 was cultured in either brain heart infusion (BHI) agar broth or agar under anaerobic conditions at 37 °C.

Antimicrobial screening assay. The resazurin microtiter assay procedure described by Palomino and coworkers was used for assessing antimicrobial activity.[2] Briefly, the assay was performed in either LB or BHI broth depending on the organism. Resazurin sodium salt powder was prepared at 0.01% (w/v) in distilled water and filter sterilized. Preliminary studies were carried out at a single inhibitor concentration of 250 μM. Growth controls containing no GNT, sterility controls without inoculation, and controls containing known antibiotics (chloramphenicol, kanamycin, or ampicillin)were also included. The innoculum was prepared from second passage cultures in the appropriate medium and standardized to $O.D._{600nm}$ = 1.0 and the wells inoculated with a 1:20 dilution. The plates were incubated for 4 h under either aerobic or anaerobic conditions as required. After incubation 30 μL of resazurin solution was added to each well and incubated for 30 min. A change in color from blue to pink indicated reduction of resazurin, and therefore bacterial growth. The MIC was determined as the lowest drug concentration that prevented this color change. Inhibition was quantified by taking the difference in absorbance between A_{570}nm-A_{600}nm. The lower the number, the greater the degree of inhibition. Compounds that demonstrated inhibition of microbial growth in the initial screen were selected for further analysis to assess whether growth inhibition was dependent on inhibitor concentration. Serial two-fold dilutions of each compound in 100 μL of medium were prepared directly in 96 well

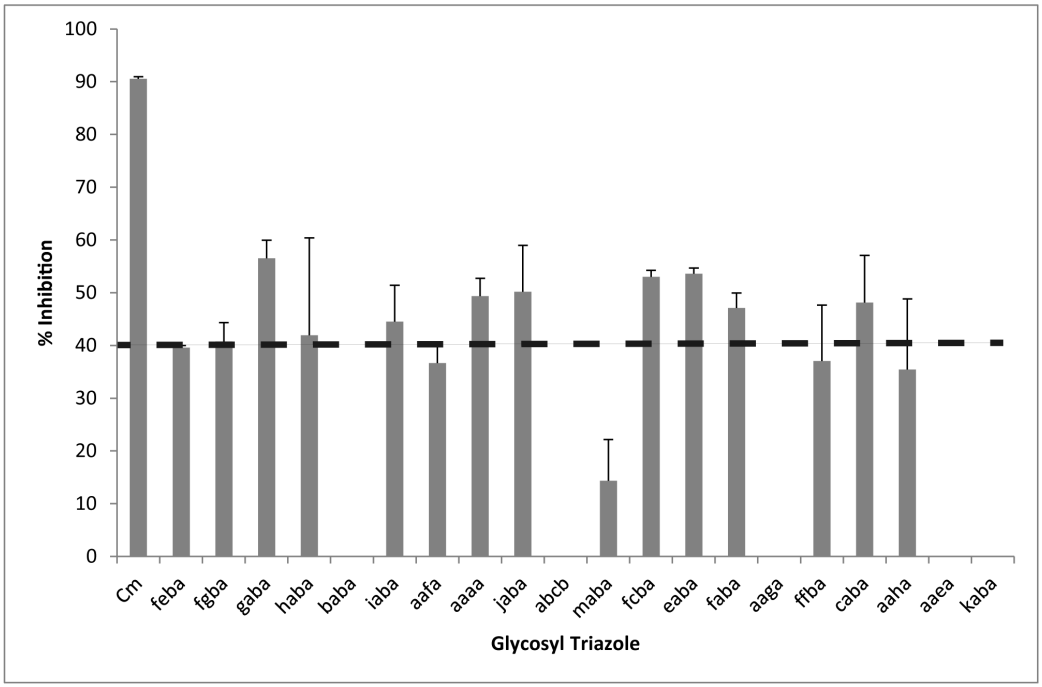
[2] Palomino, J. C.; Martin, A.; Camacho, M.; Guerra, H.; Swings, J.; Portaels, F. Antimicrobial agents and chemotherapy **2002**, *46*, 2720.

plates at concentrations ranging from 250 μM to 7 μM. Minimum inhibitory concentration was determined as the lowest drug concentration that prevented the color change from blue to pink.

Microscopy. Morphologic changes due to sub MIC concentrations of inhibitor (0.8x MIC) were monitored by phase contrast microscopy using a Zeiss Primostar phase contrast microscope equipped with an Axiocam ERc5s. All images were captured and processed using Zeiss Zen (2011 blue edition) software. Briefly, cells were grown (second passage) in the appropriate media in the presence or absence of 0.8x MIC inhibitor for 2.5 h. Cells were harvested by centrifugation (8000 rpm, 5 min), washed twice (1 mL) phosphate buffered saline and suspended in 100 μL sterile dH_2O. A 10 μL aliquot was heat fixed to a glass slide and imaged under oil immersion.

pNp-GlcNAc assay. *B. subtilis* was grown in LB broth overnight at 37 °C and the cells harvested by centrifugation (8000 rpm, 10 min). The cells were washed twice in PBS prior to re-suspending in PBS to an OD_{600}nm of 1.0. Reactions (250 μL) were set up with ***BI.fgba*** concentrations ranging from 250 μM to 0 μM. All reactions contained 4 mM pNp-GlcNAc and 198 μL of *B. subtilis* cells in PBS buffer pH 7.4. Reactions were incubated for 16 h at 30 °C prior to removal of cells by centrifugation (8,000 rpm, 10 min). Reaction supernatants were adjusted to pH 9 with the addition of 30 μL NaOH (0.5 M). Supernatants were then analyzed on a Spectromax 190 microtitre plate reader (Molecular Devices) at 490 nm. All samples were run in biological duplicate and technical triplicate.

A. *B. subtilis*



B. *B. cereus*

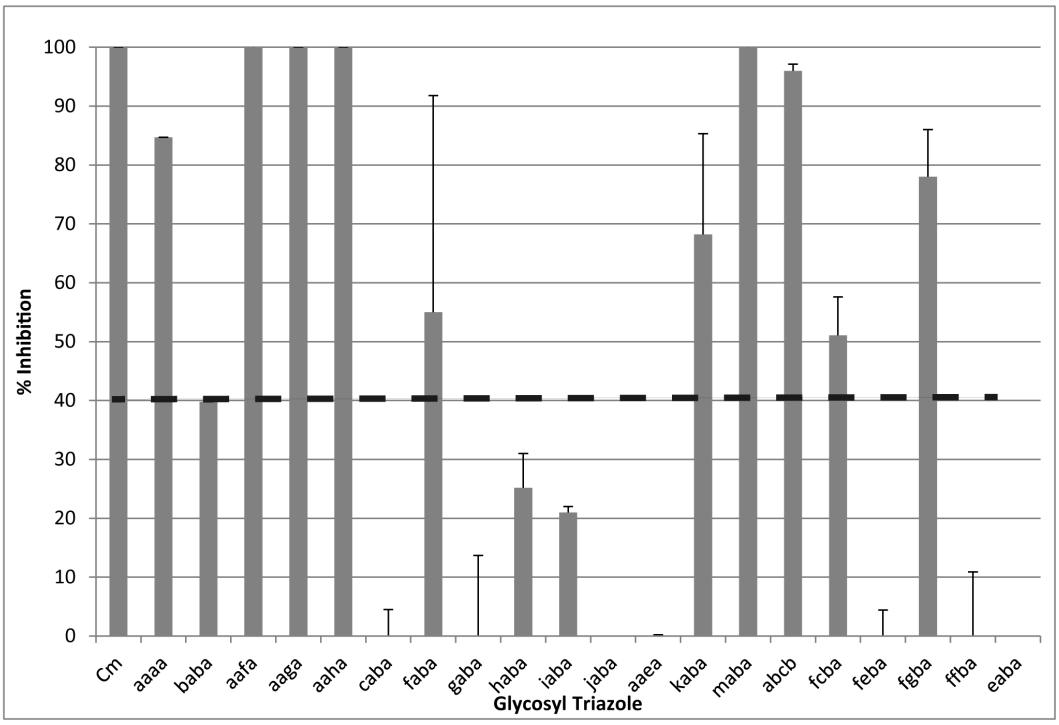


Figure S3 - % Inhibition values for GNTs against B. subtilis and cereus. The BI prefix on the compound names has been omitted for clarity. Chloramphenicol (Cm) was used as a positive control for inhibition of bacterial growth.