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Synthesis of glucose-templated lysine analogs and incorporation into the antimicrobial dipeptide sequence kW-OBn

Kaidong Zhang,[a] Dhananjoy Mondal,[a] George G. Zhanel[b] and Frank Schweizer[a,b,*]

[a]*Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2*
[b]*Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada R3E 3P4*

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Abstract—The synthesis of two glucose-templated (GlcT) lysine analogs GlcTK and GlcTk in which the side chain of D- and L-lysine (k and K) is conformationally constrained via incorporation into a D-glucose scaffold is described. A key-step in the synthesis is a high yielding, reductive ring opening of an exocyclic glucose-derived epoxide to form a α-hydroxy ester that can be converted into GlcTK and GlcTk. To demonstrate the use of these building blocks in peptide synthesis, we replaced D-lysine in the antimicrobial dipeptide sequence kW-OBn (W = L-tryptophan) and determined the antibacterial activity against various gram-positive and gram-negative organisms. Our results show that the replacement of D-lysine by unprotected GlcTk in dipeptide kW-OBn results in reduced antibacterial activity.

Keywords: Carbohydrates; Lysine; Neoglycoconjugates; Unnatural amino acids; Glycoconjugates; Glycopeptides

1. Introduction

The explosive growth of multi-drug resistant (MDR) bacteria in hospitals and the community have led to an emerging crisis where an increasing number of antimicrobial classes cease to be of clinical usefulness.[1] Despite this growing concern, only one new class of antibiotics, the oxazolidinones, has entered the clinic during the past two decades.[2] Cationic antimicrobial peptides (AMPs) with their ability to combat MDR bacteria have been proposed as new antibacterial agents.[3–5] AMPs are defined as peptides containing a net excess of positively charged residues, approximately 50% hydrophobic residues and a size ranging from 12 to 50 residues.[6]

Although the mode of action of AMPs is not fully understood, most AMPs appear to manifest their biological action by enhancing the permeability of lipid membranes of bacterial cells. This typically involves initial electrostatic interactions between the positively charged basic side chains of lysine, arginine, and ornithine to the negatively charged lipid membrane of pathogens, followed by adoption of an amphipathic α-helical or β-sheet structure.[6,7] The ability to kill target bacteria rapidly, the unusually broad spectrum of activity against some of the more serious antibiotic resistant pathogens, and the relative difficulty with which mutants develop resistance in vitro make AMPs attractive targets for drug development.[6,7]

However, in vivo efficacy studies of several cationic peptide antibiotics have been disappointing most likely due to the fact that many AMPs exhibit poor bioavailability, susceptibility to proteolytic cleavage, and low in vivo antimicrobial activity.[8] Moreover, the size of most AMPs is so large that production costs represent additional concern. To overcome some of these drawbacks, ultrashort cationic antimicrobial peptides (UAMPs) and lipopeptides in the form of di-, tri-, and tetrapeptides have recently emerged as a novel class of potential antimicrobial drug candidates.[9–11] In particular, the small size and facile preparation reduce production costs while the presence of only a few amide bonds

* Corresponding author. Tel.: +1 2044747012; e-mail: schweize@cc.umanitoba.ca

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and the low molecular weight may improve the pharmacokinetic and pharmacodynamic properties of UAMPs.

In this respect, the potent antimicrobial activity of the dipeptide D-lysine-L-tryptophan-OBn (kW-OBn) against *Staphylococcus aureus* (*S. aureus*), methicillin-resistant *S. aureus* (MRSA), and methicillin-resistant *Staphylococcus epidermidis* (MRSE) strains attracted our attention.[9] In particular, we were interested in exploring structure activity relationships of this dipeptide via replacement of D-lysine (k) by carbohydrate-templated D-lysine analogs CTLys (Fig. 1).[18] CTLys are a novel class of polyfunctional lysine analogs in which the carbohydrate scaffold constrains the side chain while at the same time introducing molecular diversity such as poly(hydroxylation) and artificial glycosylation. It is noteworthy that post-translational hydroxylation of lysine[12] and arginine[12] and other amino acids[13–15] has been shown to enhance the biological activity of AMPs.[12] Furthermore, CTLys contain the 1,3-hydroxyamine binding motifs[16] of aminoglycoside antibiotics that have been proposed to interact as bidentate RNA hydrogen bond acceptor to the phosphodiester backbone or Hoogsteen face of guanosine (Fig. 1).[16] Moreover, additional modification and derivatization of the carbohydrate scaffold may be used to tailor the physical, chemical, biological, and pharmacodynamic properties of CTLys-containing peptides.

In this paper, we report the synthesis of two glucose-templated lysines, GlcTks and GlcTKs (Fig. 1), and describe the use of these building blocks in peptide synthesis. Moreover, we replaced D-lysine (k) by GlcTk in the antimicrobial dipeptide kW-OBn and determined the antibacterial activities against various gram-positive and gram-negative bacteria. A preliminary report on the synthesis of glucose-templated lysine analogs without experimental details and biological evaluation appeared recently.[17]

2. Results and discussion

The synthesis started with the readily available D-*gluco*-configured lactone **1**[19] (Scheme 1), which reacts with the enolate of methyl bromoacetate generated from lithium bis-(trimethylsilyl)amide ($LiN(SiMe_3)_2$) in tetrahydrofuran (THF) at −78 °C, to produce the exocyclic epoxide **2** in 80% yield as a single stereoisomer.[17,20] Trimethylsilyl trifluoromethanesulfonate (TMSOTf)-promoted reductive ring opening of epoxide **2** with tributyltin hydride in dichloromethane at 0 °C afforded silylether **3** and alcohol **5** (ratio: **3**:**5** = 3.5:1) in a combined yield of 92%. Compounds **3** and **5** were obtained as a single diastereoisomer. Silyl ether **3** was hydrolyzed quantitatively into alcohol **5** by exposure to trifluoroacetic acid containing wet THF.

The stereochemistry at C-2 of alcohol **5** was determined by conversion of **5** into the known benzyl ester **4**.[21] This was achieved in a two-step procedure. First, methyl ester **5** was hydrolyzed to the corresponding acid with lithium hydroxide in wet THF. Subsequently, esterification of the acid using cesium carbonate and benzyl bromide in DMF afforded benzyl ester **4** as a single product in 89% yield. Hydroxy ester **5** served as the starting material to install an amino function at C-2. In situ activation of the hydroxyl group as a trifluoromethanesulfonate ester using trifluoromethanesulfonic anhydride in pyridine followed by nucleophilic displacement with sodium azide–15-crown-5 provided azide **6** in 82% isolated yield. Catalytic hydrogenation of **6** using Pearlman's catalyst followed by protection of the amino function as *t*-butoxycarbamate provided the protected amino ester **7** in 90% yield.

Compound **7** served as precursor for introducing the second amino functionality into the carbohydrate scaffold. This was achieved by selective tosylation[16] of the primary hydroxy group using *p*-toluenesulfonyl chloride in pyridine followed by nucleophilic displacement of the

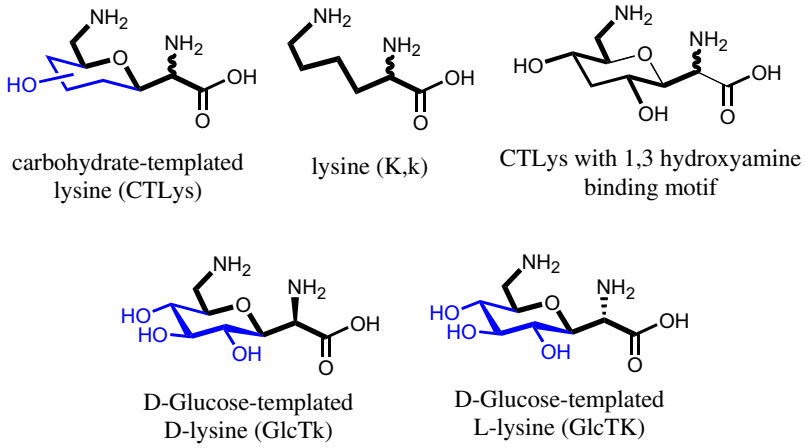
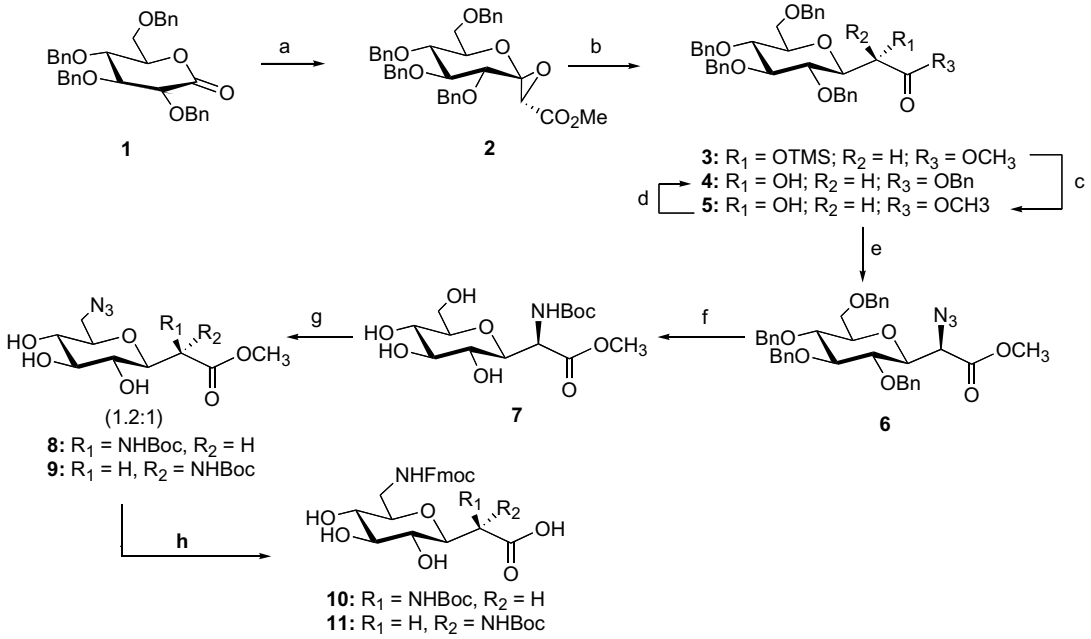


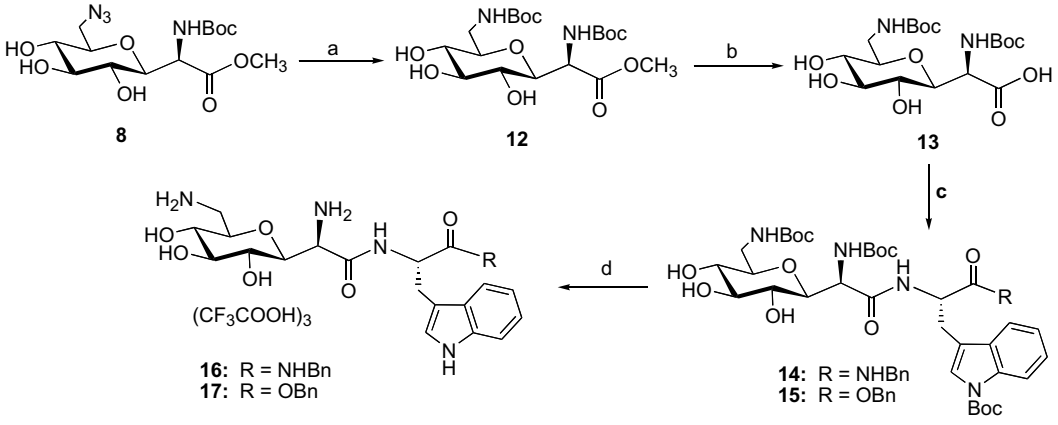
Figure 1. Examples of carbohydrate-templated lysine analogs (CTLys) and glucose-templated lysine analogs GlcTk and GlcTK.



Scheme 1. Reagents and conditions: (a) LiHMDS, $BrCH_2COOMe$, THF, −78 °C, 80%; (b) TMSOTf, Bu_3SnH, CH_2Cl_2, 0 °C, 72%; (c) CF_3COOH, THF–H_2O (5:1), rt, quant.; (d) (i) LiOH, THF–H_2O (1:1); (ii) Cs_2CO_3, BnBr, DMF, rt, 89% (over 2 steps); (e) (i) Tf_2O, dry pyridine, CH_2Cl_2, rt; (ii) NaN_3, 15-crown-5, CH_2Cl_2, rt, 81% (over 2 steps); (f) (i) $Pd(OH)_2$–C, H_2 (10 psi), MeOH, rt; (ii) $(Boc)_2O$, TEA, MeOH, 90% (over 2 steps); (g) (i) TsCl, pyridine, 0 °C to rt, 64%; (ii) NaN_3, DMF, 100 °C, 52% (**8**) and 43% (**9**); (h) (i) LiOH, THF–H_2O (1:1); (ii) $Pd(OH)_2$–C, H_2 (10 psi), MeOH, rt; (iii) Fmoc-OPfp, $NaHCO_3$, acetone–H_2O (1:1), rt, 63% (**10**, over 3 steps) or 65% (**11**, over 3 steps).

tosylate with sodium azide at elevated temperature in DMF. It is noteworthy that the displacement of the primary tosylate by sodium azide does not result in epimerization at C-2 and provides only azide **8**, if the reaction is performed below 80 °C. However, we observed substantial epimerization at 100 °C affording diastereomeric azides **8** and **9** in 53% and 42% yields, respectively. Both diastereomers can be separated by flash chromatography. Exposure of esters **8** and **9** to basic conditions (LiOH, THF–H_2O) followed by catalytic hydrogenation using Pearlman's catalyst ($Pd(OH)_2$–C, H_2, MeOH) and protection of the primary amino function as 9*H*-fluorenylmethoxycarbamate using 9-fluorenylmethyl pentafluorophenyl carbonate provided the glucose-templated D-lysine analogs GlucTk **10** and glucose-templated L-lysine analogs GlucTK **11** in 63% and 65% isolated yields, respectively. Building blocks **10** and **11** are suitably protected to be used as novel lysine mimetics in solution phase peptide synthesis.

To demonstrate the use of chimeric glucose-lysine analogs in peptide synthesis, we decided to convert azide **8** into the di-*N*-Boc-protected acid **13** (Scheme 2). This was achieved via a three-step procedure. Reduction of azide **8** by catalytic hydrogenation followed by protec-



Scheme 2. Reagents and conditions: (a) (i) $Pd(OH)_2$–C, H_2 (10 psi), MeOH, rt; (ii) Boc_2O, Et_3N, MeOH, rt (90%, over 2 steps); (b) LiOH, THF–H_2O (1:1), 0 °C, 90%; (c) TBTU, H-Trp(Boc)-NHBn, DIPEA, DMF, rt, 77% (**14**) and TBTU, H-Trp(Boc)-OBn, DIPEA, DMF, rt, 83% (**15**); (d) TFA, CH_2Cl_2, rt, quant.

tion using di-*t*-butyl dicarbonate and triethylamine in methanol afforded the di-*t*-butylcarbamate-protected ester **12** in 90% yield. Saponification of ester **12** using lithium hydroxide in aqueous THF provided acid **13** in 90% yield. To study the influence of the constrained sugar moiety and the presence of the 1,3-hydroxyamine motifs on the bioactivity of small antibacterial peptides, we decided to incorporate acid **13** into the amphiphilic antimicrobial dipeptide sequences kW-OBn[14] and kW-NHBn. The latter was selected to study how the nature of the C-terminus influences the antimicrobial activity. Amidation of **13** was achieved by the coupling of **13** to H-Trp(Boc)-NHBn and H-Trp(Boc)-OBn using 2-(1*H*-benzotriazole-1yl)-1,1,3,3-tetra-methyluronium tetrafluoroborate (TBTU) as coupling reagent in DMF to produce protected dipeptides **14** and **15** in 77% and 83% isolated yields, respectively. We did not observe ester formation involving the unprotected hydroxyl groups during peptide coupling demonstrating no need for protection of the secondary hydroxyl groups of **13** in peptide synthesis. Exposure of dipeptides **14** and **15** to a 50% mixture of trifluoroacetic acid in dichloromethane afforded unprotected dipeptides **16** and **17** in quantitative yields (Scheme 2).

With peptides **16** and **17** in hand, we investigated the antibacterial activity of these peptides against various bacterial strains and compared them to the known antimicrobial dipeptide kW-OBn **18**[9] as well as kW-NHBn **19** (Table 1). Our results show that replacement of lysine by GlcTk reduces the antibacterial activity of kW-OBn by 4–32-fold against gram-positive cocci and 2–16-fold versus gram-negative bacilli. It is possible that the low antibacterial activity of dipeptide **17** is the result of decreased amphiphilicity and hydrophobicity. For instance, substitution of D-lysine by GlcTk introduces three hydroxyl groups. This results in significantly increased hydrophilicity of GlcTk-W-OBn when compared to kW-OBn. Amphiphilicity and hydrophobicity are crucial for the antibacterial activity of AMPs.[5–7] However, other factors such as the bulkiness of the sugar fragment or the restricted rotation of the lysine motif may also contribute to the reduced activity. Interestingly, we observed that the nature of the C-terminus contributes substantially to the antimicrobial activity of kW-OBn. For instance, dipeptide kW-OBn exhibits strong *S. aureus* activity while this activity is abolished in peptide kW-NHBn. The reasons for this peculiar behavior are currently not understood.

Table 1. Representative minimal inhibitory concentrations (MIC) in μg/mL for various bacterial strains

Peptide	*S. aureus*[a]	MRSA[b]	MRSE[c]	*E. coli*[d]	*P. aeruginosa*[e]
16	256	>512	128	512	>512
17	256	256	128	512	>512
18	16	64	4	32	512
19	512	512	256	512	<512

[a] ATCC 29213.
[b] Methicillin-resistant *S. aureus* (ATCC 33592).
[c] Methicillin-resistant *S. epidermidis* (ATCC 14990).
[d] ATCC 25922.
[e] ATCC 27853.

In summary, we have developed a synthetic pathway into suitably protected glucose-templated C-glycosidic lysine analogs with natural and unnatural C(α) configuration. The carbohydrate scaffold induces a conformational constraint into the side chain of lysine while, at the same time, introducing artificial post-translational modifications such as hydroxylation and glycosylation. To demonstrate the use of carbohydrate-templated lysine analogs in peptide synthesis and to explore the biological properties, we replaced lysine in the antibacterial dipeptide kW-OBn by GlcTk. Peptide synthesis was achieved without the need for hydroxyl group protection. Determination of the biological activity against various bacterial strains demonstrated that the replacement of lysine by GlcTk in the dipeptide kw-OBn reduces strongly the antibacterial activity. We are currently studying the lysinemimetic and glycomimetic properties of GlcTk and GlcTK in other peptides.

3. Experimental

3.1. General methods

CH_2Cl_2 was distilled from calcium hydride. Organic solutions were concentrated under diminished pressure at <40 °C (bath temperature). NMR spectra were recorded at 300 or 500 MHz for ^1H and at 75 MHz for ^{13}C. Chemical shifts are reported relative to $CHCl_3$ (δ_H 7.26, δ_c (center of triplet) 77.0 ppm) or to CH_3OH (δ_H 3.35, δ_C (center of septet) 49.0 ppm) or to acetone as internal standard (D_2O). TLC was performed on E. Merck Silica Gel 60 F254 with detection by charring with 8% H_2SO_4 acid. Silica gel (0.040–0.063 mm) was used for column chromatography. Lactone **1** was purchased from Toronto Research Chemicals. Reference peptides **18** and **19** were prepared according to the literature procedure[9] and obtained as TFA salts.

3.2. (2*S*)-Methyl 2-trimethylsilyloxy-2-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-ethanoate (3) and (2*S*)-methyl 2-hydroxy-2-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-ethanoate (5)

To a mixture of epoxide **2**[21] (480 mg, 0.79 mmol) and tributyltin hydride (0.84 mL, 3.15 mmol) in CH_2Cl_2 (15 mL) was added dropwise trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.42 mL, 2.36 mmol) at 0 °C. The mixture was stirred for 30 min at this temperature and quenched with satd aq $NaHCO_3$ (15 mL). The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined

organic layers were dried (Na_2SO_4), concentrated, and purified by flash column chromatography (hexanes–EtOAc, 2:1) to afford **3** (388 mg, 72%) and **5** (96 mg, 20%) as a colorless syrup. The trimethylsilyl ether **3** was converted to **5** (337 mg, quant.) by treatment with trifluoroacetic acid (0.19 mL, 5 equiv) in aq THF (THF–H_2O, 5:1) for overnight. Compound **3**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.20 (s, 9H), 3.46–3.54 (m, 1H), 3.64 (dd, 1H, $J = 9.2$, 9.6 Hz), 3.68–3.85 (m, 8H), 4.55 (d, 1H, $J = 12.2$ Hz), 4.62 (d, 1H, $J = 12.2$ Hz), 4.63–4.74 (m, 3H), 4.86 (d, 1H, $J = 10.9$ Hz), 4.88 (d, 1H, $J = 10.9$ Hz), 4.96 (d, 1H, $J = 11.4$ Hz), 5.00 (d, 1H, $J = 11.4$ Hz), 7.22–7.41 (m, 20H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 0.9, 52.0, 68.8, 71.7, 73.2, 74.6, 75.0, 75.6, 77.7, 78.7, 79.9, 80.2, 87.4, 127.3–128.5 (aromatic carbons), 138.2, 138.5, 138.5, 138.6, 172.4; MS (ES+): m/z 707.42 $[M+Na]^+$; Anal. Calcd for $C_{40}H_{48}O_8Si$: C, 70.15; H, 7.06. Found: C, 70.25; H, 7.14; compound **5**: $[\alpha]_D^{25}$ +27.0 (c 1.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.85 (br s, 1H), 3.46–3.54 (m, 1H), 3.59 (dd, 1H, $J = 8.4$, 8.6 Hz), 3.63–3.72 (m, 3H), 3.73–3.87 (m, 5H), 4.47–4.59 (m, 3H), 4.61 (d, 1H, $J = 11.0$ Hz), 4.77 (d, 1H, $J = 11.0$ Hz), 4.85 (d, 1H, $J = 10.8$ Hz), 4.89—4.98 (m, 3H), 7.19–7.39 (m, 20H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 52.7, 69.0, 69.6, 73.3, 75.1, 75.2, 75.6, 77.8, 78.3, 79.5, 79.5, 86.9, 127.5–128.6 (aromatic carbons), 138.1, 138.2, 138.3, 138.6, 173.3; MS (ES+): m/z 635.13 $[M+Na]^+$; Anal. Calcd for $C_{37}H_{40}O_8$: C, 72.53; H, 6.58. Found: C, 72.61; H, 6.46.

3.3. (2*S*)-Benzyl 2-hydroxy-2-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-ethanoate (4)

LiOH (24 mg, 1.02 mmol) was added to a mixture of compound **5** (104 mg, 0.17 mmol) in THF–H_2O (3.0 mL, 1:1). The mixture was stirred at room temperature for 12 h and formic acid in water was added to quench the reaction until acidic. After extraction with EtOAc (5 × 20 mL), the organic layer was evaporated and the crude residue was dissolved in DMF (3.0 mL). Cs_2CO_3 (72 mg, 0.22 mmol) was added 30 min prior to the addition of BnBr (40 μL, 0.34 mmol). The mixture was stirred at room temperature for 2 h and water was added. The aqueous layer was extracted with EtOAc (3 × 20 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated. Flash column chromatography (hexanes–EtOAc, 4:1) yielded **4** (104 mg, 89%) as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.30–3.36 (m, 2H), 3.48 (dd, 1H, $J = 1.7$, 11.1 Hz), 3.59–3.64 (m, 2H), 3.67 (dd, 1H, $J = 1.9$, 8.6 Hz), 3.74 (dd, 1H, $J = 8.8$, 9.1 Hz), 3.83 (dd, 1H, $J = 9.1$, 8.6 Hz), 4.52–4.58 (m, 3H), 4.61 (d, 1H, $J = 10.7$ Hz), 4.78 (d, 1H, $J = 10.9$ Hz), 4.84 (d, 1H, $J = 10.9$ Hz), 4.90–4.97 (m, 3H), 5.10 (d, 1H, $J = 12.0$ Hz), 5.33 (d, 1H, $J = 12.0$ Hz), 7.19–7.38 (m, 25H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 67.3, 68.6, 69.6, 73.3, 75.1, 75.2, 75.6, 77.7, 78.1, 79.5, 79.6, 86.8, 127.6–128.6 (aromatic carbons), 135.3, 138.1, 138.2, 138.3, 138.6, 172.7; MS (ES+): m/z 689.44 $[M+H]^+$; Anal. Calcd for $C_{43}H_{44}O_8$: C, 74.98; H, 6.44. Found: C, 74.93; H, 6.52.

3.4. (2*R*)-Methyl 2-azido-2-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-ethanoate (6)

Compound **5** (50 mg, 0.08 mmol) was dissolved in dry CH_2Cl_2 (2.0 mL) and cooled to 0 °C. Pyridine (66 μL, 0.8 mmol) was added. The reaction mixture was stirred for 5 min, and then trifluoromethanesulfonic anhydride (55 μL, 0.32 mmol) was slowly added. The reaction mixture was stirred under 0 °C for 1 h. Water (5.0 mL) was added followed by extraction with CH_2Cl_2 (3 × 5 mL). The organic layer was dried (over Na_2SO_4), concentrated, and re-dissolved in anhyd CH_2Cl_2 (2.0 mL). NaN_3 (16 mg, 0.24 mmol) and 15-crown-5 (4 μL, 0.2 mmol) were added. The reaction mixture was stirred at room temperature for 24 h and then water was added. The aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated. Flash column chromatography (hexanes–EtOAc, 4:1) yielded azide **6** (42 mg, 81%) as a pale-yellow oil. $[\alpha]_D^{25}$ +6.0 (c 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.52–3.56 (m, 4H), 3.64 (dd, 1H, $J = 9.6$, 8.6 Hz), 3.71–3.77 (m, 3H), 3.80 (dd, 1H, $J = 9.4$, 8.6 Hz), 3.92 (dd, 1H, $J = 1.9$, 9.4 Hz), 4.37 (d, 1H, $J = 1.9$ Hz), 4.55 (d, 1H, $J = 12.1$ Hz), 4.57–4.65 (m, 2H), 4.69 (d, 1H, $J = 10.6$ Hz), 4.83 (d, 1H, $J = 10.9$ Hz), 4.88 (d, 1H, $J = 10.9$ Hz), 4.93–4.97 (m, 2H), 7.23–7.36 (m, 20H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 52.6, 62.8, 68.9, 73.5, 74.7, 75.1, 75.6, 77.3, 78.2, 79.6, 79.7, 87.1, 127.6–128.5 (aromatic carbons), 137.9, 138.0, 138.2, 138.3, 167.8; MS (ES+): m/z 660.03 $[M+Na]^+$; Anal. Calcd for $C_{37}H_{39}N_3O_7$: C, 69.68; H, 6.16; N, 6.59. Found: C, 69.72; H, 6.21; N, 6.63.

3.5. (2*R*)-Methyl 2-(*t*-butoxycarbonylamino)-2-(β-D-glucopyranosyl)-ethanoate (7)

Compound **6** (223 mg, 0.32 mmol) was dissolved in MeOH (6.0 mL) and aq hydrochloric acid (0.5 mmol). Pearlman's catalyst (160 mg) was added and the mixture was hydrogenated for 6 h at atmospheric pressure. The mixture was filtered, concentrated, and re-dissolved in MeOH (2.0 mL). Et_3N (0.5 mL) and Boc_2O (550 mg, 2.5 mmol) were added and the mixture was stirred at room temperature for 12 h. The crude residue was concentrated and purified with silica gel column chromatography (MeOH–EtOAc, 1:6) to afford **10** (100 mg, 90%) as a syrup. $[\alpha]_D^{25}$ −29.0 (c 1.45, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD): δ 1.46 (s, 9H), 3.07 (dd, 1H, $J = 9.3$, 9.2 Hz), 3.23–3.36 (m, 2H), 3.43–3.59 (m, 3H),

3.74 (s, 3H), 3.89 (dd, 1H, $J = 1.9$, 11.8 Hz), 4.66 (d, 1H, $J = <1$ Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 28.7, 52.6, 55.4, 63.6, 71.6, 72.0, 79.7, 80.8, 82.2, 82.6, 158.2, 173.0; MS (ES+): m/z 352.29 $[M+H]^+$; HRMS (ES+) calcd for $C_{14}H_{26}NO_9$ $[M+H]^+$: 352.1608; found: 352.1607.

3.6. (2*R*)-Methyl 2-(*t*-butoxycarbonylamino)-2-(6-azido-6-deoxy-β-D-glucopyranosyl)-ethanoate (8) and (2*S*)-methyl 2-(*t*-butoxycarbonylamino)-2-(6-azido-β-D-glucopyranosyl)-ethanoate (9)

To a solution of compound **7** (100 mg, 0.29 mmol) in dry pyridine (8.0 mL) was added toluenesulfonyl chloride (128 mg, 0.68 mmol) and the reaction was stirred for 2 h at 0 °C, then raised to room temperature for 6 h. The solvent was removed in vacuo and then purified by gradient flash column chromatography (EtOAc to EtOAc–MeOH, 20:1) to afford a crude mixture containing the 6-*O*-tosyl-β-D-glucopyranoside derivative (92 mg, 64%). Sodium azide (118 mg, 1.82 mmol) was added to the solution of tosylate in dry DMF (3.0 mL) and the mixture was heated to 100 °C for overnight. The solvent was removed in vacuo and the residue was purified by gradient flash column chromatography (EtOAc to EtOAc–MeOH, 20:1) to afford compounds **8** (36 mg, 52%) and **9** (30 mg, 43%) as a pale-yellow oil in a ratio 1.2:1, respectively. Compound **8**: $[\alpha]_D^{25}$ −15.0 (c 0.25, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (s, 9H), 3.32 (dd, 1H, $J = 5.9$, 13.4 Hz), 3.45 (dd, 1H, $J = 3.2$, 9.2 Hz), 3.49–3.58 (m, 4H), 3.70 (dd, 1H, $J = 3.2$, 9.2 Hz), 3.75 (s, 3H), 4.00–4.16 (br s, 1H), 4.72–4.78 (m, 2H), 4.88 (br s, 1H), 5.63 (d, 1H, $J = 8.4$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3, 51.3, 52.6, 53.9, 70.4, 70.6, 77.9, 79.5, 80.6, 80.8, 155.7, 169.6; HRMS (ES+) calcd for $C_{14}H_{24}N_4NaO_8$ $[M+Na]^+$: 399.1492; found: 399.1489; compound **9**: $[\alpha]_D^{25}$ +74.0 (c 0.1, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 9H), 3.25 (t, 1H, $J = 9.3$ Hz), 3.33 (dd, 2H, $J = 6.2$, 13.2 Hz), 3.42 (m, 2H), 3.47 (br s, 1H), 3.50 (m, 1H), 3.60 (t, 1H, $J = 8.9$ Hz), 3.76 (dd, 1H, $J = 2.3$, 9.5 Hz), 3.80 (s, 3H), 4.67 (dd, 1H, $J = 2.3$, 8.0 Hz), 4.70 (br s, 1H), 5.57 (d, 1H, $J = 8.0$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.2, 51.4, 53.0, 53.8, 69.9, 70.8, 77.2, 79.4, 80.0, 81.4, 157.4, 169.9; HRMS (ES+) calcd for $C_{14}H_{24}N_4NaO_8$ $[M+Na]^+$: 399.1492; found: 399.1490.

3.7. (2*R*)-2-(*t*-Butoxycarbonylamino)-2-[6-deoxy-6-(9*H*-fluoren-9-ylmethoxycarbonylamino)-β-D-glucopyranosyl]-ethanoic acid (10)

Ester **8** (60 mg, 0.16 mmol) was treated with lithium hydroxide (21 mg, 0.96 mmol) for 1 h at 0 °C in aqueous THF (4.0 mL, 1:1), and then acidified with formic acid (99%, 100 μL). The solution was extracted with EtOAc (6 × 10 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated to afford crude acid (58 mg, quant.), which was dissolved in MeOH (4.0 mL) and hydrogenated for 20 min using 20 wt % $Pd(OH)_2$–C. The solution was filtered and the solvent was evaporated in vacuo. The solid residue was dissolved in aqueous acetone (3.0 mL, acetone–H_2O, 1:1) and treated with 9-fluorenylmethyl pentafluorophenyl carbonate (91 mg, 0.24 mmol) and $NaHCO_3$ (31 mg, 0.37 mmol) for 4 h at room temperature. Water (10.0 mL) was added and the aqueous layer was extracted with EtOAc (6 × 10 mL). Finally, the solvent was dried (Na_2SO_4) and concentrated. The crude product was purified by flash column chromatography (MeOH–EtOAc, 1:1) to afford compound **10** (45 mg, 63%) as a colorless thick liquid. ^{1}H NMR (300 MHz, CD_3OD): δ 1.43 (s, 9H), 2.98–3.13 (m, 2H), 3.18 (m, 1H), 3.22–3.44 (m, 2H), 3.62–3.80 (m, 2H), 4.23 (t, 1H, $J = 6.8$ Hz), 4.32–4.48 (m, 3H), 7.28–7.87 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.9, 43.4, 48.5, 56.4, 67.8, 72.0, 73.1, 79.0, 80.4, 80.8, 83.0, 120.9–128.8 (aromatic carbons), 142.6–145.4 (aromatic carbons), 157.7, 159.1, 169.5; MS (ES−): m/z 557.09 $[M-H]^-$.

3.8. (2*S*)-2-(*t*-Butoxycarbonylamino)-2-[6-deoxy-6-(9*H*-fluoren-9-ylmethoxycarbonylamino)-β-D-glucopyranosyl]-ethanoic acid (11)

Ester **9** (40 mg, 0.11 mmol) was treated with lithium hydroxide (15 mg, 0.66 mmol) for 1 h at 0 °C in aq THF (4.0 mL, 1:1), and then acidified with 99% formic acid (5 drops). The solution was extracted with EtOAc (6 × 10 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated to afford crude acid (35 mg), which was dissolved in MeOH (4.0 mL) and hydrogenated for 20 min using 20 wt % $Pd(OH)_2$–C. The solution was filtered and the solvent was evaporated in vacuo. The solid residue was dissolved in aqueous acetone (3.0 mL, 2:1) and treated with 9-fluorenylmethyl pentafluorophenyl carbonate (75 mg, 0.18 mmol) and $NaHCO_3$ (30 mg, 0.36 mmol) for 4 h at room temperature. Water (10.0 mL) was added and the aqueous layer was extracted with EtOAc (6 × 10 mL). Finally, the solvent was dried (Na_2SO_4) and concentrated. The crude product was purified by flash column chromatography (MeOH–EtOAc, 1:1) to afford compound **11** (39 mg, 65%) as a thick colorless liquid. $[\alpha]_D^{25}$ +5.0 (c 0.7, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD): δ 1.45 (s, 9H), 3.13 (ABq, 1H, $J = 9.3$ Hz), 3.17–3.29 (m, 2H), 3.35 (m, 2H), 3.38–3.45 (m, 2H), 3.55–3.70 (m, 1H), 3.75 (d, 1H, $J = 9.4$ Hz), 4.20 (t, 1H, $J = 6.9$ Hz), 4.28–4.44 (m, 3H), 7.28–7.42 (m, 4H), 7.65 (dd, 2H, $J = 1.6$, 6.8 Hz), 7.78 (d, 2H, $J = 7.4$ Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 28.8, 42.7, 48.6, 56.2, 67.9, 71.2, 71.7, 78.7, 80.2, 80.8, 81.5, 120.9–128.8 (aromatic carbons) 142.6–145.4 (aromatic carbons), 158.9, 159.3, 169.2; MS (ES−): m/z 556.90 $[M-H]^-$; Anal. Calcd

for $C_{28}H_{34}N_2O_{10}$: C, 60.21; H, 6.14; N, 5.02. Found: C, 60.32; H, 6.19; N, 4.98.

3.9. (2*R*)-Methyl 2-(*t*-butoxycarbonylamino)-2-[6-deoxy-6-(*t*-butoxycarbonylamino)-β-D-glucopyranosyl]-ethanoate (12)

A solution of azide **8** (170 mg, 0.50 mmol) in MeOH (20.0 mL) was treated with $Pd(OH)_2$–C under an atmosphere of hydrogen for 1 h at room temperature. The reaction mixture was filtered through Celite, and rinsed with MeOH. The filtrate was evaporated under reduced pressure to give a colorless foam (155 mg, 0.44 mmol), which was treated with di-*t*-butyl dicarbonate (193 mg, 0.88 mmol) and Et_3N (0.3 mL, 2.2 mmol) in MeOH for 4 h at room temperature. Finally, the solvent was evaporated under reduced pressure to give a colorless liquid. The crude product was then purified by flash column chromatography (MeOH–EtOAc, 1:1) to afford compound **12** (183 mg, 90%) as a colorless liquid. $[\alpha]_D^{25}$ +4.0 (*c* 0.8, CH_3OH); ^1H NMR (300 MHz, CD_3OD): δ 1.50 (br s, 18H), 3.02 (br t, 1H, $J = 7.2$ Hz), 3.08 (br t, 1H, $J = 9.3$ Hz), 3.24 (m, 1H), 3.36 (m, 1H), 3.50 (dd, 1H, $J = 1.6$, 10.0 Hz), 3.56 (br d, 1H, $J = 9.0$ Hz), 3.64 (m, 1H), 3.78 (s, 3H), 4.69 (br s, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ 28.8, 28.9, 43.0, 52.8, 55.5, 71.7, 72.9, 79.3, 80.3, 80.9, 81.0, 82.1, 158.1, 158.7, 172.0; MS (ES+): *m*/*z* 473.24 $[M+Na]^+$; Anal. Calcd for $C_{19}H_{34}N_2O_{10}$: C, 50.66; H, 7.61; N, 6.22. Found: C, 50.54; H, 7.59; N, 6.28.

3.10. (2*R*)-2-(*t*-Butoxycarbonylamino)-2-[6-deoxy-6-(*t*-butoxycarbonylamino)-β-D-glucopyranosyl]-ethanoic acid (13)

Ester **12** (80 mg, 0.18 mmol) was dissolved in aq THF (4.0 mL, THF–H_2O, 1:1) and treated with LiOH (26 mg, 1.07 mmol) at 0 °C. The reaction mixture was stirred at this temperature for 1 h after which the mixture was acidified to pH 3 with 99% formic acid, and extracted with EtOAc (5 × 10 mL). The combined organic layers were dried (over anhyd Na_2SO_4) and concentrated under reduced pressure to give a colorless foam, which was dissolved in EtOAc (3.0 mL). Crystallization commenced within 1 h. After completion of crystallization, the solid was collected by filtration and rinsed with EtOAc (2 × 2 mL) to afford **13** (70 mg, 90%) as white crystals. Mp 205–207 °C; $[\alpha]_D^{25}$ −7.0 (*c* 0.9, CH_3OH); ^1H NMR (300 MHz, CD_3OD): δ 1.45 (s, 9H), 1.46 (s, 9H), 2.98 (m, 1H), 3.04 (t, 1H, $J = 9.2$ Hz), 3.15–3.22 (m, 1H), 3.33 (m, 1H), 3.44 (dd, 1H, $J = 1.6$, 10.0 Hz), 3.63 (br ABq, 2H, $J = 9.6$ Hz), 4.61 (br s, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ 28.7, 28.8, 43.0, 55.2, 71.8, 72.9, 79.3, 80.3, 80.8, 80.9, 82.3, 158.1, 158.7, 173.2; MS (ES+): *m*/*z* 459.23 $[M+Na]^+$; Anal. Calcd for $C_{18}H_{32}N_2O_{10}$: C, 49.53; H, 7.39; N, 6.42. Found: C, 49.49; H, 7.27; N, 6.58.

3.11. *N*-[(2*R*)-2-(*t*-Butoxycarbonylamino)-2-[6-deoxy-6-(*t*-butoxycarbonylamino)-β-D-glucopyranosyl]-acetyl]-Trp(Boc)-NHBn (14)

To the mixture of Fmoc-Trp(Boc)-OH (205 mg, 0.39 mmol) and TBTU (451 mg, 1.4 mmol) in DMF (5.0 mL) were added benzylamine (165 mL, 1.51 mmol) and *N*,*N*-diisopropylethylamine (334 μL, 1.87 mmol) and stirred for 2 h at room temperature. The solvent was removed in vacuo and the residue was purified by flash column chromatography (hexanes–EtOAc, 2:1) to yield the Fmoc-Trp(Boc)-NHBn (211 mg, 88%). The solution of Fmoc-Trp(Boc)-NHBn (151 mg, 0.25 mmol) and piperidine (0.5 mL) in DMF (2.0 mL) was stirred for 1 h at room temperature. The solvent was removed in vacuo and the crude product was purified by flash column chromatography (MeOH–EtOAc, 1:20) to afford the H-Trp(Boc)-NHBn (129 mg, 96%). To the solution of **13** (23 mg, 0.05 mmol) in DMF (2.0 mL) were added H-Trp(Boc)-NHBn (26 mg, 0.09 mmol), TBTU (58 mg, 0.18 mmol), and *N*,*N*-diisopropylethylamine (43 mL, 0.24 mmol). The mixture was stirred for 4 h at room temperature before removing the solvent under reduced pressure. The crude product was purified by flash column chromatography using EtOAc as eluent to afford **14** (29 mg, 77%) as a thick colorless liquid. $[\alpha]_D^{25}$ −5.0 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, CD_3OD): δ 1.38 (s, 9H), 1.42 (s, 9H), 1.69 (s, 9H), 2.88–3.09 (m, 2H), 3.18 (dd, 2H, $J = 7.6$, 14.2 Hz), 3.25–3.33 (m, 2H), 3.33–3.35 (m, 4H), 3.36–3.48 (m, 3H), 4.29–4.40 (m, 2H), 4.44 (br s, 1H), 4.84 (m, 1H), 7.08 (br d, 2H, $J = 7.0$ Hz), 7.20–7.37 (m, 5H), 7.52 (s, 1H), 7.66 (br d, 1H, $J = 7.6$ Hz), 8.13 (br d, 1H, $J = 8.1$ Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 28.5, 28.6, 28.8, 43.0, 44.1, 54.8, 72.0, 72.5, 79.0, 80.3, 81.0, 81.1, 84.9, 116.2, 117.2, 120.2, 123.7, 125.6, 128.2, 128.3, 129.5, 131.9, 136.9, 139.3, 151.0, 158.9, 160.6, 172.2, 173.3; MS (ES+): *m*/*z* 834.17 $[M+Na]^+$; Anal. Calcd for $C_{41}H_{57}N_5O_{12}$: C, 60.65; H, 7.08; N, 8.63. Found: C, 60.59; H, 7.07; N, 8.77.

3.12. *N*-[(2*R*)-2-(*t*-Butoxycarbonylamino)-2-[6-deoxy-6-(*t*-butoxycarbonylamino)-β-D-glucopyranosyl]-acetyl]-Trp(Boc)-OBn (15)

To a solution of Fmoc-Trp(Boc)-OH (100 mg, 0.19 mmol) in DMF (5.0 mL) was added Cs_2CO_3 (249 mg, 0.77 mmol) followed by the addition of benzyl bromide (90 μL, 0.76 mmol) and then stirred for 4 h at room temperature. The solvent was removed in vacuo and the residue was purified by flash column chromatography (hexane–EtOAc, 2:1) to yield the Fmoc-Trp(Boc)-OBn (107 mg, 92%). The solution of Fmoc-

Trp(Boc)-OBn (107 mg, 0.17 mmol) and piperidine (0.5 mL) in DMF (2.0 mL) was stirred for 1 h at room temperature. The solvent was removed in vacuo and the crude product was purified by flash column chromatography (MeOH–EtOAc, 1:20) to afford the H-Trp(Boc)-OBn (65 mg, 95%). Compound **13** (30 mg, 0.07 mmol) was dissolved in DMF (2.0 mL) and H-Trp(Boc)-OBn (36 mg, 0.09 mmol), TBTU (81 mg, 0.25 mmol), and *N*,*N*-diisopropylethylamine (60 μL, 0.34 mmol) were added and then stirred for 4 h at room temperature. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography using EtOAc as eluent to afford **15** (46 mg, 83%) as a thick liquid. $[\alpha]_D^{25}$ −32.0 (*c* 1.5, CH_3OH); ^1H NMR (300 MHz, CD_3OD): δ 1.38 (br s, 18H), 1.63 (s, 9H), 3.00–3.15 (m, 2H), 3.18 (d, 2H, $J = 7.0$ Hz), 3.24 (br s, 1H), 3.33–3.40 (m, 5H), 4.89 (m, 1H), 5.03 (s, 2H), 7.06–7.12 (m, 2H), 7.15–7.31 (m, 5H), 7.40 (br s, 1H), 7.50 (m, 1H), 8.05 (d, 1H, $J = 8.2$ Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 26.9, 27.3, 27.4, 27.6, 40.8, 54.6, 54.9, 66.7, 69.8, 70.1, 76.8, 77.3, 78.8, 79.0, 79.7, 83.4, 114.7, 114.8, 118.2, 122.2, 123.4, 124.0, 127.4, 127.8, 127.9, 134.5, 134.9, 149.3, 155.4, 157.1, 170.0, 171.3; MS (ES+): *m*/*z* 835.33 $[M+Na]^+$; Anal. Calcd for $C_{41}H_{56}N_4O_{13}$: C, 60.58; H, 6.94; N, 6.89. Found: C, 60.57; H, 6.88; N, 6.93.

3.13. *N*-[(2*R*)-2-(Amino)-2-[6-deoxy-6-(amino)-β-D-glucopyranosyl]-acetyl]-Trp-NHBn·$(CF_3CO_2H)_3$ (16)

A solution of TFA–CH_2Cl_2 (1:1, 4.0 mL) was added to compound **14** (80 mg, 0.10 mmol) at room temperature. After 3 h, the solution was diluted with toluene (2 × 5 mL), concentrated in vacuo, provided **16** (73 mg, quant.) as a salt. $[\alpha]_D^{25}$ −8.0 (*c* 0.45, CH_3OH); ^1H NMR (300 MHz, D_2O): δ 2.52–2.74 (m, 2H), 2.88–3.04 (m, 2H), 3.28–3.40 (m, 2H), 3.42–3.56 (m, 2H), 3.70–3.77 (m, 1H), 4.32–4.41 (m, 1H), 4.45–4.54 (m, 2H), 5.00–5.11 (m, 1H), 7.16–7.49 (m, 8H), 7.60–7.68 (m, 1H), 7.78 (dd, 1H, $J = 7.8$, 17.8 Hz); ^{13}C NMR (75 MHz, D_2O): δ 27.9, 40.9, 43.4, 53.4, 54.8, 69.4, 70.8, 76.0, 76.6, 77.4, 109.0, 112.6, 118.9, 119.7, 122.3, 125.1, 126.8, 127.4, 127.8, 129.1, 136.8, 137.8, 166.1, 173.3; MS (ES+): *m*/*z* 511.99 $[M+H]^+$.

3.14. *N*-[(2*R*)-2-(Amino)-2-[6-deoxy-6-(amino)-β-D-glucopyranosyl]-acetyl]-Trp-OBn·$(CF_3CO_2H)_3$ (17)

Compound **15** (100 mg, 0.12 mmol) was treated with a solution of TFA–CH_2Cl_2 (1:1, 4.0 mL) at room temperature for 3 h. The volatiles were removed with toluene (2 × 5 mL) in vacuo and then the residue was rinsed with dry ether to afford **17** (91 mg, quant.) as a TFA salt. $[\alpha]_D^{25}$ −16.0 (*c* 0.3, CH_3OH); ^1H NMR (300 MHz, D_2O): δ 2.32–2.42 (m, 2H), 2.70–2.78 (t, 1H, $J = 9.6$ Hz), 3.18 (br d, 1H, $J = 14.0$ Hz), 3.22–3.42 (m, 3H), 3.48–3.60 (m, 2H), 4.30 (br s, 1H), 5.14 (dd, 1H, $J = 5.7$, 10.3 Hz), 5.24 (br s, 2H), 7.17 (t, 1H, $J = 7.6$ Hz), 7.22 (br s, 1H), 7.28 (t, 1H, $J = 7.6$ Hz), 7.36 (dd, 2H, $J = 3.8$, 7.6 Hz), 7.42–7.49 (m, 3H), 7.53 (d, 1 H $J = 8.2$ Hz), 7.70 (d, 1H, $J = 8.2$ Hz); ^{13}C NMR (75 MHz, D_2O): δ 27.2, 40.9, 53.3, 53.6, 68.4, 69.3, 70.7, 76.0, 76.5, 77.5, 109.1, 112.6, 118.9, 119.7, 122.4, 125.0, 126.7, 128.7, 129.1, 129.2, 135.4, 136.8, 166.2, 173.3; MS (ES, $[M+Na]^+$); MS (ES+): *m*/*z* 513.23 $[M+H]^+$.

3.15. Determination of the MIC values for peptides 16–19

Bacterial isolates were obtained from the American Type Culture Collection (ATCC). Isolates were kept frozen in skim milk at −80 °C until minimum inhibitory concentration (MIC) testing was carried out. Following two subcultures from frozen stock, the in vitro activities of peptides were determined by macrobroth dilution in accordance with the Clinical and Laboratory Standards Institute (CLSI) 2006 guidelines.[22] Stock solutions of peptides were prepared and dilutions made as described by CLSI. Test tubes contained doubling antimicrobial dilutions of cation adjusted Mueller–Hinton broth and inoculated to achieve a final concentration of approximately 5×10^5 CFU/mL, then incubated in ambient air for 24 h prior to reading. Colony counts were performed periodically to confirm inocula. Quality control was performed using ATCC QC organisms.

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