Synthesis of Sugar–Lysine Chimera with Integrated *gluco*-Configured 1,3-Hydroxyamine Motif

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Abstract: The paper describes the synthesis of glucose-configured sugar–lysine chimeras in which the side chain of lysine is conformationally constrained via incorporation into a D-glucose scaffold. Key step in the synthesis is a high-yielding, reductive ring opening of an exocyclic glucose-derived epoxide to form an α-hydroxyester that can be converted into chimeric sugar–lysine analogues. To demonstrate the use of these novel chimeric sugar–lysine building blocks in peptide coupling, we replaced D-lysine in the antimicrobial dipeptide sequence kW by a D-glucose–D-lysine chimera.

Key words: sugar–lysine chimera, reductive ring opening, epoxides

Amino acids containing basic side chains (lysine, ornithine and arginine) occur frequently in antimicrobial peptides (AMPs). 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 pathogenic cells. This typically involves initial electrostatic interactions between the positively charged basic side chains to the negatively charged lipid membrane of pathogens, followed by adoption of an amphipathic α-helical or β-sheet structure.[1,2] Although more potent antibiotics exist, the ability to kill target cells rapidly, unusually broad activity spectra against some of the more serious antibiotic resistant pathogens and relative difficulty with which mutants develop resistance in vitro make AMPs attractive targets for drug development.[1,2] However, in vivo studies of many cationic peptide antibiotics have been disappointing most likely due to the fact that many AMPs exhibit poor bioavailability, susceptibility to proteolytic cleavage and low antimicrobial activity.[3] In order to overcome some of these drawbacks novel approaches are in high demand. This paper describes the synthesis of sugar–lysine chimeras (SlysC, **1**, Figure 1). GlcLysCs are molecules in which a cyclic C-glycosidic scaffold becomes part of, or mimics, the regular side chain of lysine. The cyclic nature of the carbohydrate constrains the side chain of lysine while the polyfunctional nature of the scaffold introduces chemical diversity and artificial post-translational modifications such as hydroxylation and glycosylation. It is noteworthy that post-translational hydroxylation of lysine,[4] arginine[4] and other amino acids[5–7] has enhanced the biological activity of AMPs.[4] In addition, post-translational glycosylation of the antimicrobial peptide drosocin[8,9] provided analogues with increased antibacterial activity. In order to study the effect of artificial hydroxylation and glycosylation of lysine in antibacterial peptides we describe here the synthesis of *gluco*-configured, conformationally constrained GlcLysC analogues **1** with natural or unnatural configurations at the α-carbon. Compound **1** contains the *gluco*-configured RNA 1,3-hydroxyamine binding motif[10] of aminoglycoside antibiotics that has been proposed to interact as bidentate RNA hydrogen bond donor to the phosphodiester backbone or Hoogsteen face of guanosine (Figure 2). Incorporation of **1** into short antibacterial peptides may introduce novel and synergistic effects, which could improve the biological, pharmacological and chemical properties of antibacterial peptides.

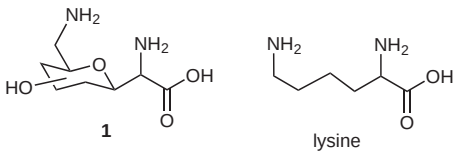

Figure 1 Structure of a sugar–lysine chimera (SLysC) **1**.

The synthesis started with the readily available D-*gluco*-configured lactone **2**[11] (Scheme 1) which reacts with the enolate of α-bromo acetic acid methylester generated from lithium bis(trimethylsilyl)amide [$LiN(SiMe_3)_2$] in THF at –78 °C, to produce the exocyclic epoxide **3** in 80% yield as a single stereoisomer.[12] Trimethylsilyltrifluoromethanesulfonate (TMSOTf)-promoted reductive ring opening of epoxide **3** with tributyltin hydride in dichloromethane at 0 °C afforded a mixture containing silylether **4** and alcohol **5** (ratio **4**:**5** = 3.5:1) in a combined yield of 88%. Compounds **4** and **5** were obtained as a single diastereoisomer. The silylether **4** 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 benzylester **6**.[13] This was achieved in a two-step procedure. At first, methylester **5** was hydrolyzed to the corresponding acid with lithium hydroxide in wet THF. Subsequently, esterfication of the acid using cesium carbonate and benzylbromide in DMF afforded benzylester **6** as a single product in 89% yield. Hydroxyester **5** served as starting material to install an amino function at C-2. Initially, we activated the hydroxyl group as a tri-

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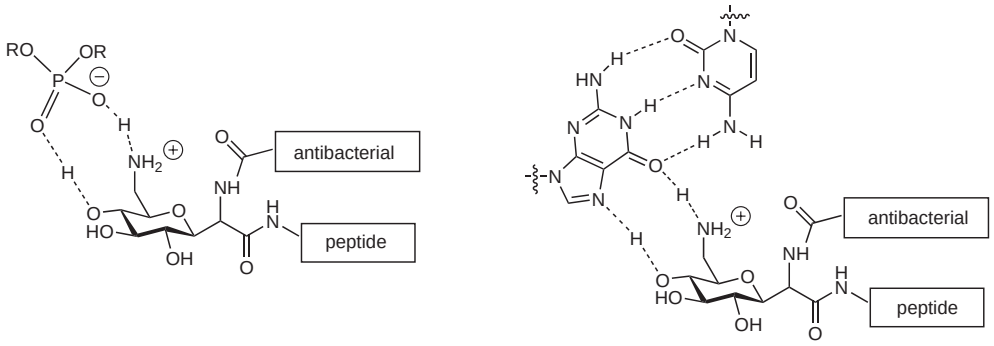
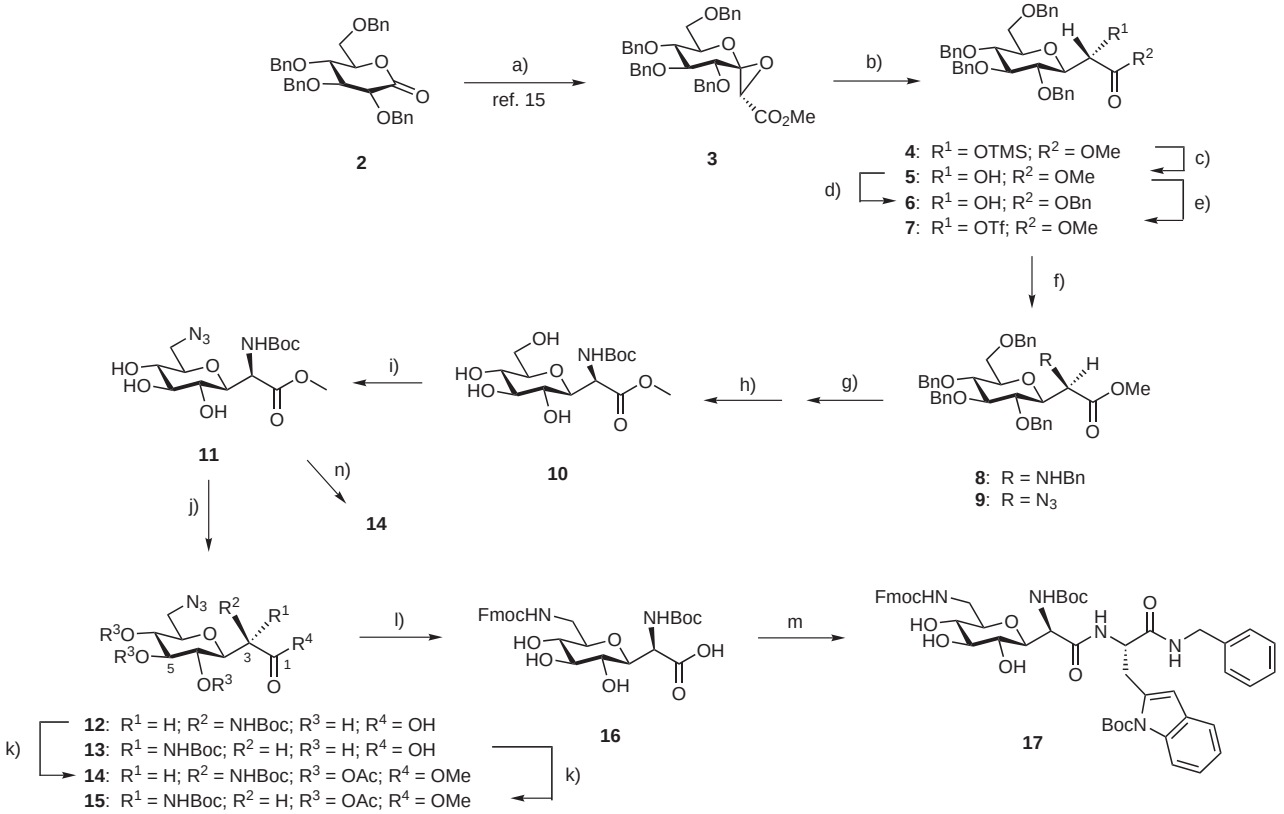


Figure 2 Proposed mechanism of an antibacterial peptide containing a 1,3 hydroxyamine binding motif for RNA recognition. Phosphodiesters and the Hoogsteen face of guanosine represent bidentate RNA hydrogen bond acceptors that may be recognized by hydroxyamines (modified from ref. 10).

fluoromethanesulfonate ester **7** using trifluoromethane sulfonic anhydride in pyridine. Subsequently, we studied the displacement reaction of triflate **7** with two nucleophiles benzylamine and sodium azide. Both reactions provided the corresponding amino ester **8** and azide **9** in 56% and 81% yield, respectively. Catalytic hydrogenation of **8** and **9** using Pearlman's catalyst followed by protection of the amino function as *tert*-butyloxycarbamate provided protected amino ester **10** in 90% yield. Compound **10** served as starting material to introduce a second amino function into the carbohydrate scaffold. This was achieved by selective tosylation of the primary hydroxy position using *p*-toluenesulfonic chloride in pyridine followed by nucleophilic displacement of the tosylate with sodium azide to produce compound **11** in 65% isolated yield. Exposure of ester **11** to basic conditions (LiOH, THF–H_2O) resulted in partial epimerization at the C-2 position, affording an inseparable mixture of acids **12** and **13**. Acids **12** and **13** were converted into the epimeric sugar–lysine hybrids **14** and **15** (ratio **14**:**15** = 4:1) by



Scheme 1 *Reagents and conditions*: (a) LiHMDS, $CH_2BrCOOMe$, THF, –78 °C to r.t., 2 h, 80%; (b) Bu_3SnH, TMSOTf, CH_2Cl_2, 0 °C, 30 min, 88%; (c) THF, TFA (2 equiv) 2 h quant.; (d) (i) LiOH (3.0 equiv), THF, H_2O, 12 h; (ii) Cs_2CO_3, BnBr, DMF, 4 h, 89%; (e) Tf_2O, pyridine, CH_2Cl_2, 0 °C 1 h, quant.; (f) NaN_3, CH_2Cl_2, 15-crown-5, r.t., 24 h, 81% or $BnNH_2$, CH_2ClCH_2Cl, 50 °C, 24 h, 56%; (g) $Pd(OH)_2$, MeOH, HCl (1.3 equiv), quant.; (h) MeOH, Boc_2O (2 equiv), Et_3NH, 12 h, 90%; (i) TsCl (2.2 equiv), pyridine, r.t., 12 h; then work-up and NaN_3, DMF, 80 °C, 12 h, 65%; (j) LiOH (5 equiv), THF, H_2O, 8 h; (k) (i) Cs_2CO_3, DMF, MeI work-up; (ii) Ac_2O, pyridine, 12 h, quant.; (l) $Pd(OH)_2$, H_2, MeOH, 30 min then FmocOPfp (2 equiv), $NaHCO_3$ (4 equiv), r.t., acetone–H_2O (3:1), 2 h, 63%; (m) TBTU, H-Trp(Boc)-NHBn, DIEA, DMF, 80%; (n) Ac_2O–pyridine.

esterfication (Cs_2CO_3, MeI, DMF) followed by acetylation of the hydroxyl groups (Ac_2O, pyridine). At this stage it was possible to separate the epimeric diastereomers by flash chromatography. The major diastereomer was identical to compound **14** previously obtained by acetylation of ester **11**. Compounds **14** and **15** exhibit characteristic ^{1}H NMR data that confirm their structure. For instance, compound **14** shows the expected downfield shifts of H-4, H-5 and H-6 ($\delta_{H4,H5,H6} > 5.00$ ppm) that are characteristic for O-acetylation at C-4, C-5 and C-6. By comparison, protons H-8a and H-8b in compound **15** appear high field ($\delta_{H8a,b} = 3.20–3.33$ ppm), clearly indicating the installation of the azido function at C-8. In addition, the observed vicinal coupling constant between H-3 and H-4 of 9.3 Hz demonstrates the diaxial relationship of these protons and proves that no epimerization occurred at C-3 during treatment with LiOH. Analogously, the epimeric sugar–lysine hybrid **15** exhibits chemical shifts and vicinal diaxial coupling constants ($\delta_{H4,H5,H6} > 5.00$ ppm; $J_{H3,H4} = 9.9$ Hz) that confirm its C-2 epimeric structure.

To demonstrate the use of GlcLysCs in peptide coupling reactions we decided to convert azido acid **14** into Fmoc-protected amino acid **16**. This was achieved by catalytic hydrogenation followed by selective protection of the amino function using 9-fluorenylmethyl pentafluorophenyl carbonate (FmocOPfp) to produce **16** in 63% isolated yield. The lysine analogue **16** is orthogonally protected to be used in solution-phase peptide coupling. To study the influence of the constrained sugar moiety and the presence of the *gluco*-configured 1,3-hydroxyamine motif on the bioactivity of small antibacterial peptides, we decided to incorporate GlcLysC into the amphiphilic antimicrobal dipeptide sequence kW.[14] This was achieved by coupling of **16** to H-Trp(Boc)-NHBn using 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetra-methyluronium tetrafluoroborate (TBTU) as coupling reagent in DMF to produce dipeptide **17** in 80% isolated yield. During this coupling we did not observe ester formation as evidenced by MS analysis of the crude product or exposure to basic conditions (K_2CO_3, MeOH) as previously reported by Knorr et al.[15]

In summary, we have developed a synthetic pathway into suitably protected C-glycosidic glucose–lysine chimeras with natural and unnatural C(α) configuration that can be used in peptide coupling reactions without need for hydroxyl group protection. The carbohydrate scaffold induces conformational constraint into the side chain of lysine while, at the same time, introducing artificial post-translational modifications such as hydroxylation and glycosylation. Furthermore, the SLysC (**1**) incorporates the RNA-recognizing *gluco*-configured putative 1,3-hydroxyamine motif which may introduce synergistic effects when incorporated into antibacterial peptides. We are currently studying the lysinemimetic and glycomimetic properties of **1** in small peptides as well as the antimicrobial properties of **17**.[16]

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(16) **Synthetic Procedures for Compounds 12–17.**
Synthesis of Compounds 12–15.
Ester **11** (60 mg, 0.16 mmol) was treated with LiOH (7 mg, 0.31 mmol) for 8 h at r.t. in aq THF (1:1), and then acidified with formic acid (100 μL). The solution was extracted with EtOAc (6 × 10 mL) and the combined organic layer solvent was dried (Na_2SO_4) and concentrated to afford inseparable mixture of crude acids **12** and **13** (59 mg, quant.), which was treated with Cs_2CO_3 (61 mg, 0.18 mmol) and MeI (30 μL, 0.48 mmol) in DMF. The reaction was worked up with H_2O and extracted with EtOAc (4 × 15 mL); the combined organic phases were dried (Na_2SO_4) and concentrated. The crude was acetylated by dissolving it in a 1:1 mixture containing Ac_2O (0.5 mL) and pyridine (0.5 mL). The crude mixture was purified by the flash chromatography (EtOAc–hexane, 1:2) to afford compound **14** (61 mg, 80%) and **15** (15 mg, 20%). Compound **14** was identical to the product obtained by acetylation of compound **11**.
Synthesis of Compound 16.
Acid **12** (46 mg, 0.12 mmol) was dissolved in MeOH (4 mL) and hydrogentated for 20 min using 20 wt% Pd/C. The solution was filtered and the solvent was evaporated in vacuo. The solid residue was dissolved in aq acetone (3 mL, 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 r.t. Then, H_2O (10 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 **16** (45 mg, 63%).
Synthesis of Compound 17.
To the mixture of Fmoc-Trp(Boc)-OH (205 mg, 0.39 mmol) and benzylamine (165 μL, 1.51 mmol) in DMF (5 mL) was added TBTU (249 mg, 0.77 mmol) and DIPEA (340 μL, 1.95 mmol). The reaction was stirred for 2 h at r.t. The solvent was removed in vacuo and the residue was purified

by flash column silica gel chromatography (2:1, hexane–EtOAc) to yield the Fmoc-Trp(Boc)-NHBn (151 mg, 63%). The solution of Fmoc-Trp(Boc)-NHBn (151 mg, 0.25 mmol) and piperidine (0.5 mL) in DMF (2 mL) was stirred for 1 h at r.t. The solvent was removed in vacuo and the crude product was purified by flash column silica gel chromatography (from EtOAc to 5% MeOH in EtOAc) to afford the NH_2-Trp(Boc)-NHBn (81 mg, 80%). Compound **16** (23 mg, 0.04 mmol) was dissolved in DMF (2 mL) and NH_2-Trp(Boc)-NHBn (72 mg, 0.18 mmol), TBTU (33 mg, 0.10 mmol), and DIPEA (37 μL, 0.21 mmol). The mixture was stirred for 4 h at r.t. before removing the solvent under reduced pressure. The crude product was purified by flash chromatography using EtOAc as eluent to afford **17** (24 mg, 63%).

Characteristic Spectroscopic Data for Compounds 14–17.

Compound **14**: ^{1}H NMR (300 MHz, $CDCl_3$, r.t., TMS): δ = 1.45 (s, 9 H), 2.00 (s, 3 H), 2.02 (s, 3 H), 2.05 (s, 3 H), 3.20–3.33 (m, 2 H), 3.64–3.70 (m, 1 H, H-7), 3.77 (s, 3 H), 3.87 (dd, 1 H, H-3, *J* = 9.9, 1.7 Hz), 4.66 (dd, 1 H, H-2, *J* = 9.2, 1.7 Hz), 5.02 (dd, 1 H, H-6, *J* = 9.3, 9.7 Hz), 5.16 (dd, 1 H, H-5, *J* = 9.3, 9.1 Hz), 5.25 (dd, 1 H, H-4, *J* = 9.9, 9.1 Hz), 5.46 (d, 1 H, N–H, *J* = 9.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, r.t.): δ = 20.57, 20.58, 20.66, 28.27 (3 C), 50.83, 52.73, 53.47, 68.77, 69.03, 74.21, 77.77, 78.76, 80.46, 168.78, 168.80, 169.39, 169.51, 170.29. MS (ES): *m*/*z* calcd for $C_{20}H_{30}N_4NaO_{11}$ $[M + Na]^+$: 525.18; found: 525.32. Anal. Calcd (%) for $C_{20}H_{30}N_4O_{11}$: C, 47.81; H, 6.02; N, 11.15. Found: C, 48.08; H, 6.15; N, 10.77.

Compound **15**: ^{1}H NMR (300 MHz, $CDCl_3$, r.t., TMS): δ = 1.44 (s, 9 H), 1.98 (s, 3 H), 2.03 (s, 3 H), 2.04 (s, 3 H), 3.13–3.29 (m, 2 H, H-8a, H-8b), 3.64–3.71 (m, 1 H, H-7), 3.78 (s, 3 H), 4.16 (dd, 1 H, H-3, *J* = 9.9, 2.3 Hz), 4.70 (dd, 1 H, H-2, *J* = 10.5, 2.3 Hz), 5.00 (dd, 1 H, H-6, *J* = 9.5, 9.5 Hz), 5.06 (dd, 1 H, H-4, *J* = 9.9, 9.5 Hz), 5.20 (dd, 1 H, H-5, *J* = 9.5, 9.5 Hz), 5.13 (d, 1 H, N–H, *J* = 10.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, r.t.): δ = 21.23, 21.34 (2 C), 28.88 (3 C), 51.30, 53.02, 53.46, 68.12, 69.84, 74.59, 78.04, 78.47, 79.82, 167.63, 168.12, 168.84, 169.37, 169.72. MS (ES): *m*/*z* calcd for $C_{20}H_{30}N_4NaO_{11}$ $[M + Na]^+$: 525.18; found: 525.27. Anal. Calcd (%) for $C_{20}H_{30}N_4O_{11}$: C, 47.81; H, 6.02; N, 11.15. Found: C, 47.98; H, 6.25; N, 11.55.

Compound **16**: ^{1}H NMR (300 MHz, CD_3OD, r.t., TMS): δ = 1.43 (s, 9 H), 2.98–3.13 (m, 2 H, H-6 and H-8a), 3.18 (m, 1 H, H-**7**), 3.22–3.44 (m, 2 H, H-3, H-5), 3.62–3.80 (m, 2 H, H-4, H-8b), 4.23 (t, 1 H, *J* = 6.8 Hz), 4.32–4.48 (m, 3 H), 7.28–7.87 (m, 8 H). ^{13}C NMR (100 MHz, $CDCl_3$, r.t.): δ = 28.88, 43.42, 48.54, 56.37, 67.78, 72.04, 73.14, 79.03, 80.38, 80.76, 82.97, 120.94–128.78 (arom. C), 142.61–145.43 (arom. C), 157.71, 159.12, 169.46. MS (ES): *m*/*z* calcd for $C_{28}H_{33}N_2O_{10}$ $[M - H]^-$: 557.21; found: 557.09.

Compound **17**: ^{1}H NMR (300 MHz, $CDCl_3$, r.t., TMS): δ = 1.38 (s, 9 H), 1.63 (s, 9 H), 3.04–3.34 (m, 6 H), 3.37–3.61 (m, 5 H), 3.71–3.83 (m, 1 H), 4.03–4.37 (m, 5 H), 4.45 (dd, 1 H, *J* = 6.7, 2.2 Hz), 4.75–4.85 (m, 1 H), 5.45–5.56 (br s, 1 H, NH), 6.12–6.29 (br s, 1 H, NH), 6.29–6.46 (br s, 1 H, NH), 6.73–6.91 (m, 2 H), 6.96–7.03 (br, 1 H, NH), 7.08–8.18 (m, 16 H). HRMS (ES): *m*/*z* calcd for $C_{51}H_{59}N_5NaO_{12}$ $[M + Na]^+$: 956.40524; found: 956.40537.