

## Supporting Information

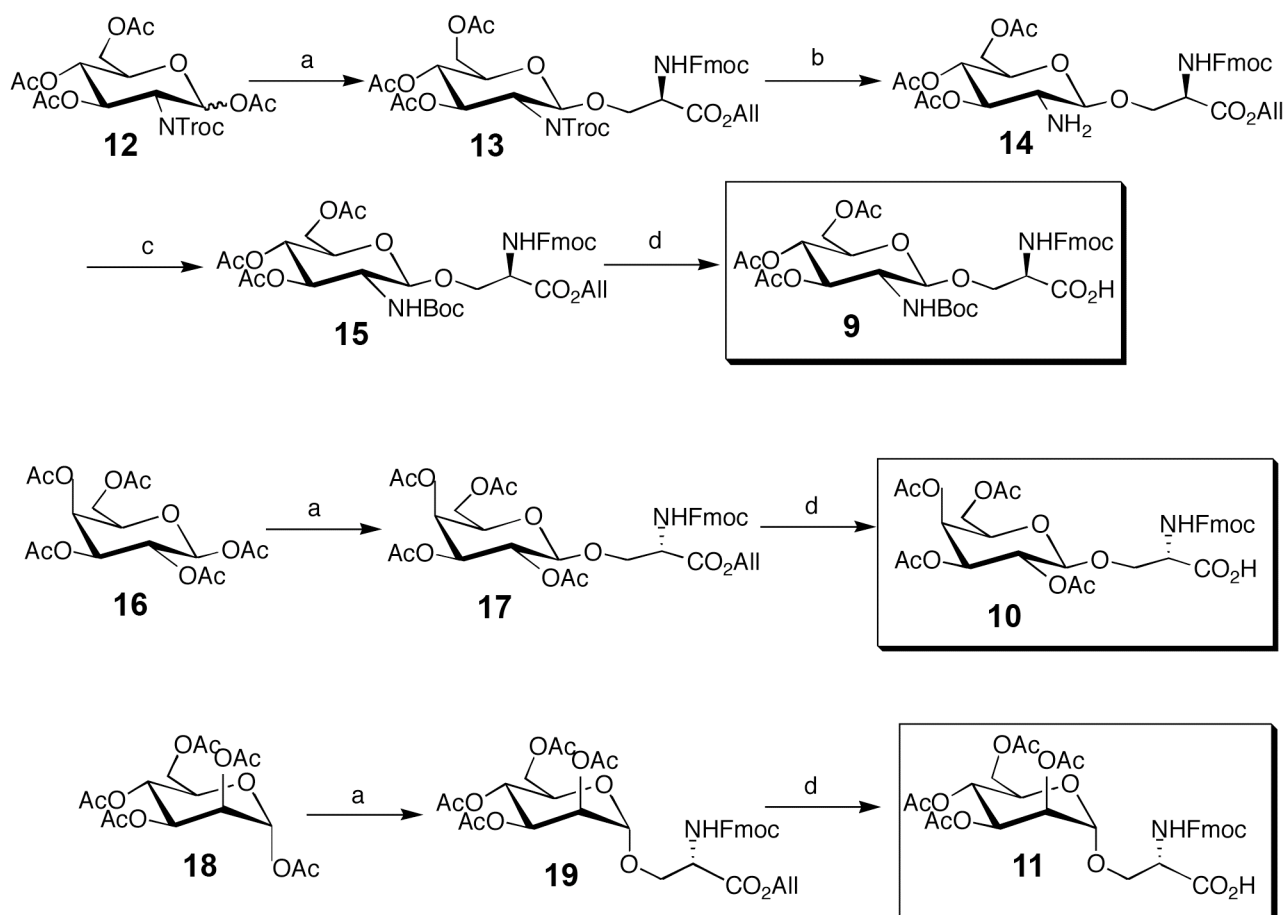
### Antibacterial Cyclic D,L- $\alpha$ -Glycopeptides

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**General.** 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylserine (POPS), and 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC) were purchased from Avanti. MP-TMT, and 7-Azabenzotriazol-1-yloxy-tris(pyrrolidino) phosphonium hexafluorophosphate (PyAOP) were purchased from Argonaut and Applied Biosystems, respectively. Anhydrous dichloromethane and dioxane were obtained from Acros. All other chemicals were obtained from Aldrich, Acros, Novabiochem or Sigma and used without further purification. TLC was run on SiO<sub>2</sub> 60F<sub>254</sub> (Merck) and detected with UV and anisaldehyde reagent. All non-aqueous reactions were carried out in oven-dried glassware under an inert Ar atmosphere. NMR spectra were recorded on Bruker AMX-500 MHz spectrometer.

**Scheme S1.** Synthesis of building blocks **9**, **10**, and **11**.



(a): Fmoc-Ser-OAll, BF<sub>3</sub>.OEt<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>; (b) Zn, AcOH; (c) Boc<sub>2</sub>O, TEA; (d) Pd(PPh<sub>3</sub>)<sub>4</sub>, Morpholine

Fmoc-D-Ser-OAll and Fmoc-L-Ser-OAll were synthesized according to published procedures.<sup>S1</sup>

**Fmoc-D-Ser ( $\beta$ Ac<sub>3</sub>GlcNH<sub>2</sub>Boc)-OH (9):**

Synthesis of **12** from D-Glucosamine in two steps has been previously reported.<sup>S2</sup>

**Fmoc-D-Ser( $\beta$ GlcNTroc)-OAll (13).**<sup>S3</sup> Fmoc-D-Ser-OAll (367 mg, 1.0 mmol) and **12** (522 mg, 1.0 mmol) were first azeotroped with dry toluene, and then dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (5 mL) under Ar. The solution was cooled on an ice bath, and BF<sub>3</sub>.OEt<sub>2</sub> (250  $\mu$ L, 2 mmol) was added dropwise. The reaction was stirred for 4 hours at room temperature, then additional portion of glycosyl donor **12** (522 mg, 1.0 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (2 mL) and BF<sub>3</sub>.OEt<sub>2</sub> (250  $\mu$ L, 2 mmol) were added at 0°C, and stirring were continued for 24 h at room temperature. The solution was diluted with CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and washed with 0.5 N KHSO<sub>4</sub> (1 $\times$ 20 mL) and water (1 $\times$ 15 mL). The organic layer was dried over MgSO<sub>4</sub> and concentrated in *vacuo*. The residue was purified by chromatography (n-Hex/EtOAc, 4:1) to afford white foam in 75% yield. <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>)  $\delta$  7.77 (t, *J* = 8 Hz, 2H), 7.63 (t, *J* = 7.5 Hz, 2H), 7.41 (dd, *J* = 4.5, 11.5 Hz, 2H), 7.33 (dd, *J* = 6, 13.5 Hz, 2H), 5.89 (m, 2H), 5.33 (brd, *J* = 16.5 Hz, 1H), 5.26 (d, *J* = 10 Hz, 1H), 5.21 (t, *J* = 10 Hz, 1H), 5.05 (d, *J* = 9 Hz, 1H), 4.99 (t, *J* = 10 Hz, 1H), 4.83 (d, *J* = 12 Hz, 1H), 4.64 (brd, *J* = 5.5 Hz, 2H), 4.52 (dd, *J* = 6.5, 10.5 Hz, 1H), 4.45 (dd, *J* = 6.5, 10.5 Hz, 2H), 4.38 (d, *J* = 8 Hz, 1H), 4.23 (t, *J* = 6 Hz, 1H), 4.13 (m, 2H), 4.02 (m, 2H), 3.62 (dd, *J* = 8.5, 19 Hz, 1H), 3.50 (m, 2H), 2.05, 2.0, 1.98 (3s, total 9H).

**Fmoc-D-Ser( $\beta$ Ac<sub>3</sub>GlcNH<sub>2</sub>)-OAll (14).** A suspension of activated zinc (5.2 g, 79.5 mmol) in a solution of **13** (4 g, 4.82 mmol) in glacial acetic acid (35 mL) was stirred for 6 hours. TLC showed completion, and the reaction was filtered through Celite<sup>®</sup>. The solvent was removed and azeotroped with hexane. The residue was dissolved in dichloromethane (40 mL) and washed with saturated NaHCO<sub>3</sub>, and brine. The aqueous layer was extracted with dichloromethane (10 mL). The combined organic layers were dried over MgSO<sub>4</sub>, and concentrated in *vacuo* to yield **14** (2.85 g, 90 %). This compound was used for the next step without further purification.

**Fmoc-D-Ser( $\beta$ Ac<sub>3</sub>GlcNH<sub>2</sub>Boc)-OAll (15).** To a solution of glycoside **14** (2.3 g, 3.5 mmol) and Boc<sub>2</sub>O (1.2 g, 4.5 mmol) in dry dioxane (20 mL) triethylamine (1.5 mL, 10.7 mmol) was added dropwise under Ar. After 16 h of stirring at room temperature, the dioxane was removed under vacuum. The resulting residue was dissolved in ethyl acetate (50 mL), washed with saturated NaHCO<sub>3</sub>, and water. The organic layer was dried over MgSO<sub>4</sub> and concentrated in *vacuo*. Chromatography (n-Hex/EtOAc, 7:3) afforded an amorphous solid in 65% yield. <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>)  $\delta$  7.77 (t, *J* = 8 Hz, 2H), 7.62 (d, *J* = 7.0 Hz, 2H), 7.40 (dd, *J* = 7.5, 17.5 Hz, 2H), 7.32 (dd, *J* = 7.5, 11.5 Hz, 2H), 5.94-5.87 (m, 2H), 5.33 (d, *J* = 17.5 Hz, 1H), 5.25 (d, *J* = 10 Hz, 1H), 5.19 (brs, 1H), 4.98 (t, *J* = 9.5 Hz, 1H), 4.65 (t, *J* = 5 Hz, 1H), 4.54-4.40 (m, 5H), 4.23 (t, *J* = 6.5 Hz, 2H), 4.17-4.09 (m, 2H), 4.01 (brt, *J* = 14 Hz, 2H), 3.52 (m, 2H), 2.07, 2.05, 1.98 (3s, total 9H), 1.44, 1.43 (2s, 9H).

**Fmoc-D-Ser( $\beta$ Ac<sub>3</sub>GlcNH<sub>2</sub>Boc)-OH (9).** Compound **15** (754 mg, 1.0 mmol) and Pd(PPh<sub>3</sub>)<sub>4</sub> (11.5 mg, 0.01 mmol) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (8 mL), and morpholine (165  $\mu$ L, 1.9 mmol) was added dropwise under Ar. After stirring for 30 min, the solution was diluted with CH<sub>2</sub>Cl<sub>2</sub> (8 mL), washed with 10% citric acid (4 mL) and brine (4 mL), dried over MgSO<sub>4</sub> and concentrated. Residual palladium was scavenged using MP-TMT resin [0.04-0.05 equiv. relative to Pd (PPh<sub>3</sub>)<sub>4</sub>] for a period of 16-24 hours. After treatment with MP-TMT, the building block **9** isolated by filtration of the resin followed by solvent removal (yield = 95%). <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>)  $\delta$  7.76 (d, *J* = 7.5 Hz, 2H), 7.63 (t, *J* = 7.5 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.0 Hz, 2H), 5.74 (brs, 1H), 5.24 (brs, 1H), 5.02 (t, *J* = 10 Hz, 1H), 4.56 (brs, 1H), 4.41 (m, 3H), 4.29 (m, 2H), 4.23 (t, *J* = 7 Hz, 2H), 4.05 (d, *J* = 10.5 Hz, 2H), 4.03 (brs, 1H), 3.59 (m, 2H), 2.06, 2.02, 1.99 (3s, total 9H), 1.49, 1.44, 1.41 (3s, total 9H). FAB HRMS: 715.2695 ([M+H]<sup>+</sup>, calc, 715.2714), 737.2528 ([M+Na]<sup>+</sup>, calc, 737.2534).

**Fmoc-L-Ser(Ac<sub>4</sub>Gal)-OH (10).** Fmoc-Ser-OAll (367 mg, 1.0 mmol) and glycosyl donor **16** (390 mg, 1.0 mmol) were first azeotroped with dry toluene, and then dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (5 mL) under Ar. The solution was cooled on an ice bath, and BF<sub>3</sub>.OEt<sub>2</sub> (250  $\mu$ L, 2 mmol) was added dropwise. The reaction was stirred for 4 h at room temperature, then additional portion of glycosyl donor **16** (390 mg, 1.0 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (2 mL) and BF<sub>3</sub>.OEt<sub>2</sub> (250  $\mu$ L, 2 mmol) were added at 0°C, and stirring were continued for 24 h at room temperature. The solution was diluted with CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and washed with 0.5 N KHSO<sub>4</sub> (1 $\times$ 20 mL) and water (1 $\times$ 15 mL). The organic layer was dried over MgSO<sub>4</sub> and concentrated in *vacuo*. The residue was purified by column chromatography (n-Hex/EtOAc, 4:1) to afford **17** in 78% yield. <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>)  $\delta$  7.76 (dd, *J* = 3.5, 7.5 Hz, 2H), 7.61 (dd, *J* = 4.2, 6.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.33 (m, 2H), 5.90 (m, 1H), 5.58 (d, *J* = 8 Hz, 1H), 5.37 (dd, *J* = 1.0, 2.5 Hz, 1H), 5.33 (d, *J* = 17.5 Hz, 1H), 5.27 (dd, *J* = 1.0, 10.0 Hz, 1H), 5.16 (dd, *J* = 8.0, 10.5 Hz, 1H), 4.99 (dd, *J* = 3.5, 10.5 Hz, 2H), 4.67 (d, *J* = 5.5 Hz, 2H), 4.50 (m, 2H), 4.43 (m, 2H), 4.29 (dd, *J* = 3.0, 11 Hz, 1H), 4.24 (t, *J* = 8 Hz, 1H), 4.10 (d, *J* = 7 Hz, 2H), 3.89 (dd, *J* = 3.5, 7.5 Hz, 1H), 3.84 (t, *J* = 6 Hz, 2H), 2.14,

2.05, 2.03, 1.99 (4s, total 12H). FAB HRMS: 698.2444 ( $[M+H]^+$ , calc, 698.2449), 720.2258 ( $[M+Na]^+$ , calc, 720.2268).

Compound **17** (697 mg, 1.0 mmol) and Pd(PPh<sub>3</sub>)<sub>4</sub> (11.5 mg, 0.01 mmol) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (8 mL), then morpholine (165  $\mu$ L, 1.9 mmol) was added dropwise under Ar. After stirring for 30 min., the solution was diluted with CH<sub>2</sub>Cl<sub>2</sub> (8 mL), washed with 10% citric acid (4mL) and brine (4mL), dried over MgSO<sub>4</sub> and concentrated. Residual of palladium was scavenged using MP-TMT resin [0.04-0.05 equiv. relative to Pd (PPh<sub>3</sub>)<sub>4</sub>] for a period of 16-24 hours. After treatment with MP-TMT, **10** is isolated by filtration of the resin followed by solvent removal (yield 93%). <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>)  $\delta$  7.76 (brd,  $J=7.5$  Hz, 2H), 7.61 (brt,  $J=6$  Hz, 2H), 7.40 (t,  $J=7.5$  Hz, 2H), 7.32 (m, 2H), 5.64 (d,  $J=8$  Hz, 1H), 5.38 (d,  $J=3.5$  Hz, 1H), 5.17 (t,  $J=9$  Hz, 1H), 5.01 (dd,  $J=3.0, 10.0$  Hz, 1H), 4.51 (m, 2H), 4.44 (m, 2H), 4.30 (dd,  $J=2.5, 11$  Hz, 1H), 4.23 (t,  $J=7$  Hz, 1H), 4.16 (dd,  $J=6.5, 11.5$  Hz, 1H), 4.10 (dd,  $J=6.5, 11.5$  Hz, 1H), 3.94 (dd,  $J=3.0, 10.5$  Hz, 1H), 3.84 (t,  $J=7$  Hz, 1H), 2.14, 2.03, 2.01, 1.99 (4s, total 12 H). FAB HRMS: 658.2125 ( $[M+H]^+$ , calc, 658.2136), 680.1945 ( $[M+Na]^+$ , calc, 680.1955).

**Fmoc-L-Ser ( $\alpha$ Ac<sub>4</sub>Man)-OH (11)**. Compound **18** was reacted according to the procedure described in the synthesis of **10**.

**Fmoc-L-Ser ( $\alpha$ Ac<sub>4</sub>Man)-OAll (19)**. Yield 76%. <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>)  $\delta$  7.76 (d,  $J=7.5$  Hz, 2H), 7.63 (d,  $J=7$  Hz, 2H), 7.39 (t,  $J=7.5$  Hz, 2H), 7.33 (t,  $J=7.5$  Hz, 2H), 5.93 (m, 1H), 5.87 (d,  $J=8.0$  Hz, 1H), 5.36 (d,  $J=17.0$  Hz, 1H), 5.30-5.23 (m, 3H), 5.20 (brs, 1H), 4.81 (s, 1H), 4.76-4.67 (m, 2H), 4.60 (m, 1H), 4.41 (d,  $J=7.5$  Hz, 2H), 4.24 (m, 2H), 4.11 (m, 1H), 4.08 (dd,  $J=2.5, 10.5$  Hz, 1H), 3.99 (m, 2H), 2.15, 2.04, 2.02, 1.97 (4s, total 12H). FAB HRMS: 698.2446 ( $[M+H]^+$ , calc, 698.2449), 720.2268 ( $[M+Na]^+$ , calc, 720.2268).

**Fmoc-L-Ser ( $\alpha$ Ac<sub>4</sub>Man)-OH (11)**. Yield 95%. <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>)  $\delta$  7.75 (d,  $J=7.5$  Hz, 2H), 7.60 (t,  $J=7.5$  Hz, 2H), 7.38 (t,  $J=7.5$  Hz, 2H), 7.29 (t,  $J=7.0$  Hz, 2H), 6.48 (d,  $J=8.0$  Hz, 1H), 5.42 (dd,  $J=3.0, 10.0$  Hz, 1H), 5.26 (m, 2H), 4.86 (s, 1H), 4.67 (m, 1H), 4.42 (dd,  $J=7.5, 10.5$  Hz, 1H), 4.32 (dd,  $J=7.5, 10.5$  Hz, 1H), 4.25 (d,  $J=5.5$  Hz, 1H), 4.21 (dd,  $J=6, 15$  Hz, 2H), 4.05 (m, 4H), 2.17, 2.15, 2.05, 2.0 (4s, total 12 H). FAB HRMS: 658.2126 ( $[M+H]^+$ , calc, 658.2136), 680.1945 ( $[M+Na]^+$ , calc, 680.1955).

### General Procedure for Peptide Synthesis.

(a) Loading of resin: a solution of Fmoc-D-Lys-OAll (1.25 mmol) and diisopropylethylamine (4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (12 mL) was added to 2-chlorotrityl chloride resin (1.0 mmol, 1.2 mmol/g loading max) for 6 hours. Final loading was quantified by UV quantitation of Fmoc release, and was found to be 0.65 mmol/g.

(b) Peptide Synthesis: The linear peptides were synthesized using an automated synthesizer (Advanced ChemTech, 348  $\Omega$ ). Coupling reactions were run on a 0.05-mmol scale using standard Fmoc protocol. The coupling was carried out using six-fold excess of each amino acid (coupling for 90 min), except for glycosylated amino acids (2-fold excess, coupling for 3 h), HOBT/DIC as coupling reagents and 25% piperidine in NMP for Fmoc deprotection. After removal of the *N*-terminal Fmoc, the resin was exposed to Pd(PPh<sub>3</sub>)<sub>4</sub> (0.2 equiv.) and PhSiH<sub>3</sub> (10 equiv.) in CH<sub>2</sub>Cl<sub>2</sub> for 5h to remove the *C*-terminal allyl protecting group. Residual palladium was removed by washing the resin with 1% solution of sodium dimethyldithiocarbamate hydrate in DMF. Cyclization was then performed using PyAOP/HOBT/DIEA (4:4:8 equiv.) for 5 h and HOBT/DIC (4:4 equiv.) for overnight. Following deprotection of acetyl groups by 20% hydrazine in MeOH for 2  $\times$  2.5 h, the peptides were cleaved from resin by TFA/H<sub>2</sub>O/triisopropylsilane (95:2.5:2.5) for 2 h. The crude peptides were purified by preparative RP-HPLC on a C<sub>18</sub> column.

**Liposome Fluorescence Assays.** LUVs were prepared by the reverse evaporation method from 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC, 11 mg), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC, 10 mg), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylserine (POPS, 1 mg), and cholesterol (6 mg) in 4.8 mL HEPES buffer (pH 7.2) containing 100 mM sulforhodamine B. The vesicles were sized by repeated extrusion through a stack of 0.4- and 0.2  $\mu$ m-pore size polycarbonate membranes, and then unincorporated dye was removed via gel filtration on Sephadex G-25 in HEPES pH 7.2 buffer. The final assay cuvette contained 1 mL of the HEPES buffer (pH 7.2), 12.5  $\mu$ L of the prepared vesicle suspension (9.5 mM in lipids and cholesterol), and 10  $\mu$ L of the peptide (1, 0.5, 0.25, 0.125 mM in DMSO) to give a final approximate peptide: lipid ratio of 1:11.5, 1:23, 1:46, 1:92 respectively.

Fluorescence intensity data was collected every second at 585 nm (excitation at 535 nm). Data were normalized for comparison into fractional change in fluorescence given by  $(I_0 - I_t)/(I_0 - I_f)$  where  $I_0$  is the initial,  $I_f$  the final fluorescence intensity after addition of the detergent triton X-100, and  $I_t$  the observed fluorescence intensity, respectively.

**Peptide/Lipid Multibilayer Sample Preparation for IR.** Vesicles were prepared from 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC) by the reverse-phase evaporation method. Briefly, to a solution of 20 mg of DMPC in 1:1 volume of chloroform/ether, 2 mL of ddH<sub>2</sub>O was added. The suspension was sonicated to yield a milky white solution, and then the organic solvents were rotovapped off to yield the aqueous vesicle suspension. The peptide solution (10 mM in DMSO) was added to the vesicle suspension diluted 1:1 by volume with ddH<sub>2</sub>O, to yield a final peptide: lipid molar ratio of 1:9 and DMSO content of less than 8% in the final volume. The mixture was vortexed vigorously, allowed to incubate at room temperature for 15-20 min, then gel-filtered through a fine-grade Sephadex G-25 column to remove the nonincorporated peptide. The filtrate was applied onto the germanium crystal. The lipid/peptide film was allowed to orient by air-drying at room temperature without disturbance.

**MIC, MBC, HD<sub>50</sub>, and LD<sub>50</sub> measurements.** All measurements were carried out as previously reported.<sup>S4</sup>

### References:

- (S1) Kriek N. M. A. J.; Filippov, D. V.; van den Elst H.; Meeuwenoord, N. J.; Tesser, G. I.; van Boom, J. H.; van der Marel, G. A.; *Tetrahedron* **2003**, *59*, 1589-1597.  
 (S2) (a) Autar R.; Khan S.; Schad M.; Hacker J.; Liskamp M. J.; Pieters R. J.; *ChemBioChem* **2003**, *4*, 1317-1325. (b) Dullenkopf W.; Castro-Palomino J. C.; Manzoni L.; Schmidt R. R.; *Carbohydr. Res.* **1996**, *296*, 135-147.  
 (S3) Saha U. K.; Schmidt R. R.; *J. Chem. Soc. Perkin Trans 1*, **1997**, 1855-1860.  
 (S4) Dartois, V., Sanchez-Quesada, J., Cabezas, E., Chi, E., Dubbelde, C., Dunn, C., Granja, J., Gritzen, C., Weinberger, D., Ghadiri, M. R., and Parr, T. R., Jr. *Antimicrob. Agents Chemother.* **2005**, *49*, 3302-3310.

**Table S1.** ATR FT-IR data and calculated orientation of cyclic peptide nanotubes in DMPC lipid multibilayers.

Cyclic peptide	Frequency (cm <sup>-1</sup> )		Peptide		Lipid	
	Amide-A	Amide-I	[A <sub>  </sub> /A <sub>⊥</sub> ] <sup>a</sup>	Tilt <sup>c</sup>	[A <sub>  </sub> /A <sub>⊥</sub> ] <sup>b</sup>	Tilt <sup>c</sup>
2 [W <sub>L</sub> W <sub>K</sub> S <sub>S</sub> X <sub>S</sub> K <sub>L</sub> ]	3278	1629	1.42	64°	1.20	30°
4 [W <sub>L</sub> W <sub>K</sub> S <sub>S</sub> K <sub>Z</sub> K <sub>L</sub> ]	3276	1627	1.42	64°	1.17	29°
6 [W <sub>L</sub> W <sub>K</sub> S <sub>S</sub> K <sub>U</sub> K <sub>L</sub> ]	3275	1626	1.17	69°	1.12	27°

<sup>a,b</sup> Dichroic ratios of the amide-I band<sup>a</sup> or antisymmetric CH<sub>2</sub> stretches of DMPC lipid ( $\nu_{as}(\text{CH}_2) = 2919 \text{ cm}^{-1}$ )<sup>b</sup> intensities with parallel polarized incident light to the corresponding band intensities with perpendicular polarized light. <sup>c</sup> Tilt refers to the angle of the molecular axis with respect to the surface normal. Lipid and peptide nanotube tilt angles are calculated according to the methods detailed in [8]. <sup>d</sup> Difference between the angles of the peptide tube axis and the lipid hydrocarbon chain. X = Ser( $\beta$ GlcNH<sub>2</sub>), Z = Ser( $\beta$ Gal), and U = Ser( $\alpha$ Man).