Design, Synthesis, and Evaluation of a Liposaccharide Drug Delivery Agent: Application to the Gastrointestinal Absorption of Gentamicin

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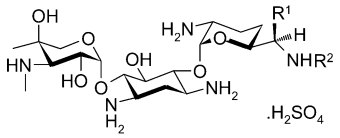
The design, synthesis, and evaluation of a liposaccharide (**11**) for use as an agent to enhance the gastrointestinal absorption of charged, hydrophilic drugs with poor membrane permeability is reported. **11** was designed to possess both surfactant and ion-pairing properties and was conveniently synthesized from D-glucuronic acid (**2**) and *N*-Boc-lipoamino acid (**5**) precursors in eight steps in good yield. Isothermal titration microcalorimetry was used to determine the critical micelle concentration of **11** (in PBS) to be 2.09 ± 0.01 mM with an enthalpy of demicellization of 4.91 ± 0.11 kJ/mol. The ability of **11** to enhance the gastrointestinal absorption of the aminoglycoside antibiotic gentamicin (**1**), a hydrophilic polycation with negligible oral bioavailability, was assessed in vivo using rats. Rats dosed orally with a mixture of **11** (100 mg/kg) and **1** (60 mg/kg) had a statistically significant ($P \leq 0.034$) increase in C_{max}, AUC_{120}, and percent absolute bioavailability (F) compared to control **1** (60 mg/kg) alone. The highest bioavailability ($F = 9.1 \pm 2.0\%$) was achieved by dosing with the mixture **11** (100 mg/kg) and **1** (15 mg/kg). This represents a 6-fold increase in bioavailability compared to the control ($F = 1.4 \pm 0.3\%$). These results suggest that the molar ratio of **1**:**11** may be critical in optimizing the delivery system, a finding ascribed in part to the ion-pairing properties of **11**. The effect of **11** on the gastrointestinal mucosa was assessed using light microscopy to examine tissue samples from rats used in the pharmacokinetic study. No morphological changes were found in either the esophagi or duodena of the rats examined. One rat dosed with **11** (100 mg/kg) and **1** (60 mg/kg) exhibited slight gastric erosion, which could be attributed to **11**.

Introduction

Many therapeutic drugs exhibit poor membrane permeability with subsequent low oral bioavailability and must therefore be delivered via the parenteral route. Compared to oral delivery, parenteral therapy has many disadvantages, including greater risk of adverse events, patient discomfort, higher drug product cost, additional cost of equipment, and time and expertise needed for administration.[1,2] Consequently, research has focused on developing methods to overcome poor membrane permeability of pharmaceuticals. Formulation of a drug with penetration enhancing excipients, such as surfactants or ion-pairing agents, represent methods by which membrane permeability can be increased.[3,4] In this paper we describe the design, synthesis, and evaluation of a liposaccharide excipient that combines the properties of surfactants and ion-pairing agents. For evaluation purposes, we chose the aminoglycoside antibiotic gentamicin as a representative drug with poor membrane permeability and hence low oral bioavailability.[5,6]

The aminoglycosides are among the drugs of choice for any suspected Gram-negative bacteremic infection with a spectrum that includes virtually all Gram-negative bacteria that are not strict anaerobes.[7] Structurally, aminoglycosides consist of two or more amino sugars joined via glycosidic linkages to a cyclohexyl nucleus, which is usually in a central position.[6] Specifically, the gentamicins C_1, C_2, and C_{1a}, which constitute the gentamicin complex (**1**), contain a central deoxystreptamine unit linked to two amino saccharides, garosamine and a variable purpurosamine unit.[8,9] The presence of multiple amino and hydroxyl groups makes these molecules hydrophilic polycations, which explains their poor gastrointestinal absorption. In fact, less than 1% of a dose is absorbed following either oral or rectal administration.[6,10]



1

	R^1	R^2
Gentamicin C_1	CH_3	CH_3
Gentaminin C_2	CH_3	H
Gentamicin C_{1a}	H	H

Efforts have been made to develop aminoglycosides as oral pharmaceutical products. For instance, Recchia and co-workers concluded that a semisynthetic saponin had potential as a transmucosal delivery agent for the aminoglycosides.[11] The saponin was more effective at

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drug transport than the structurally similar penetration enhancers sodium glycocholate and glycyrrhizic acid. Similarly, a glycosteroid was effective in the transport of gentamicin when it was admixed with the drug and administered either into the ileal or jejunal intestinal segments of rats and dogs, or by oral gavage to dogs.[12] Compared with the bile acid taurocholate, the glycosteroid was significantly better as an absorption enhancer. Banerjee et al. achieved the oral delivery of tobramycin in mice by using the vehicle CRL-1605, a nonionic block copolymer synthesized from ethylene oxide and propylene oxide.[13] Most recently, Hu et al. used a product obtained from coconut oil, Labrasol, which contains saturated polyglycolysed C6−C14 glycerides, as an agent to enhance gastrointestinal absorption of gentamicin in rats.[14,15] All of the excipients in these studies are surfactants.

This study reports the use of a liposaccharide (**11**) to enhance the oral absorption of gentamicin (**1**). Like the excipients detailed above, **11** is a surfactant. In addition, it is a lipophilic anion, designed to ion-pair with **1** and promote passive membrane diffusion of a drug−liposaccharide complex.

Ion-pairs may be defined as neutral species formed by electrostatic attraction between oppositely charged ions in solution, which are often sufficiently lipophilic to dissolve in nonaqueous solvents.[4] The first to utilize ion-pairing as a means of drug delivery was Irwin and co-workers in 1969.[16] Following Irwin's success, much research focused on ion-pairing to enhance the absorption of drugs, and the reader is referred to reviews of the topic.[4,17,18] Evidence of the ability of **1** to complex with anionic materials is shown in its isolation from fermentation broth using cationic-exchange resin[5] and in its binding to the acidic mucopolysaccharide heparin.[19]

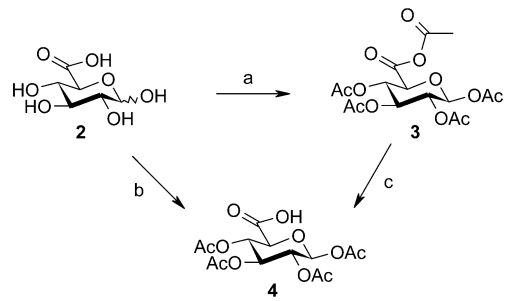
Details of the design, synthesis, and characterization of liposaccharide **11** as an agent for drug delivery follow.

Results and Discussion

Synthesis. Anionic liposaccharide **11** was conveniently synthesized from lipoamino acid (Laa) and D-glucuronic acid (GlcA) precursors. Lipoamino acids (Laas) conjugated to drugs have proved successful in improving drug oral absorption.[20−22] An advantage of using Laa moieties in excipients is the ability to modulate the physicochemical properties of the system by varying the Laa alkyl chain length, a feature dependent on the alkyl bromide used in Laa synthesis.[23,24] Laas are poorly water soluble, so to increase the aqueous solubility, GlcA was conjugated with the Laa via an amide linkage.[24,25] The sugar forms part of a putative surfactant polar headgroup and masks the amino functionality of the Laa, leaving a carboxylic acid as an anionic group for ion-pairing to the cationic **1**. The nature of the sugar moiety has been shown to affect the particulate-forming properties of sugar−lipid conjugates.[24,25]

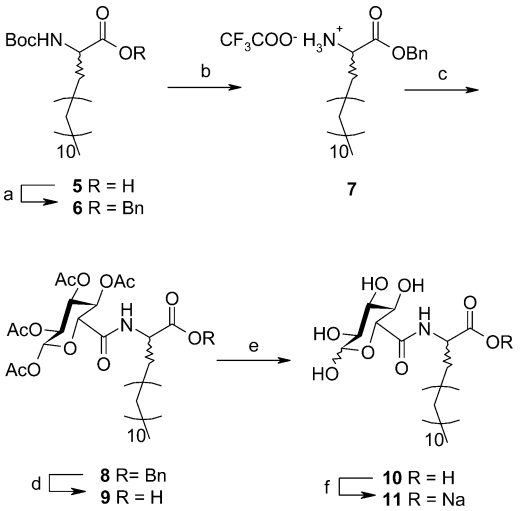
Following the published procedure,[26] reaction of GlcA (**2**) with acetic anhydride in the presence of iodine, followed by treatment with methanol in one pot, gave the peracetylated glucuronic acid (**4**), despite reports to the contrary (Scheme 1).[27] In an alternative approach, **4** was obtained in two steps via isolation of the anhydride (**3**), which was then converted to **4** by stirring in wet THF.[27,28]

Scheme 1[a]



[a] Reagents and conditions: (a) Ac_2O, I_2, 0 °C to 25 °C, 18 h; (b) (i) Ac_2O, I_2, 2 h at 0 °C then 1 h at 25 °C, (ii) MeOH, 1 h at 0 °C then 18 h at 25 °C; (c) H_2O/THF, 25 °C, 18 h.

Scheme 2[a]



[a] Reagents and conditions: (a) (i) Cs_2CO_3, MeOH, (ii) BnCl, DMF, 70 °C, 4 h; (b) CF_3COOH, DCM, 1 h; (c) **4**, HBTU, DIEA, THF, 18 h; (d) H_2, 10% Pd/C, THF, 18 h; (e) (i) 0.1 M NaOMe, MeOH, 2 h, (ii) Amberlite IR-120 (H^+); (f) $NaHCO_3$.

The Boc-protected Laa (**5**) was obtained via minor modification to the literature procedure (Scheme 2).[23] Conversion of **5** to the benzyl ester (**6**), followed by removal of the Boc protecting group by treatment with TFA, gave **7**. The lipid (**7**) and sugar (**4**) moieties were conjugated using HBTU/DIEA activation to afford **8**. Subsequent removal of the benzyl group of **8** by hydrogenolysis, followed by deacetylation with sodium methoxide in methanol solution, afforded the liposaccharide (**10**). To facilitate ion-pairing and increase the aqueous solubility, the free acid (**10**) was converted to the sodium salt (**11**) by treatment with 1 equiv of sodium bicarbonate in water.

Critical Micelle Concentration. The micellization of Laas conjugated with hydrophilic compounds such as lactic, glycolic, and gluconic acids was studied by ^1H NMR using organic solvents.[25] GlcA/Laa conjugates structurally similar to **11** were shown to form liposomes when mixed with dimyristol lecithin alone or in combination with cholesterol.[24] No quantification of the critical micelle concentration (cmc) of sugar/Laa conjugates has been reported for aqueous systems. The cmc is an important physicochemical parameter that can help to explain the interaction of an amphiphile with a biological system. Glycosylated and nonglycosylated bile

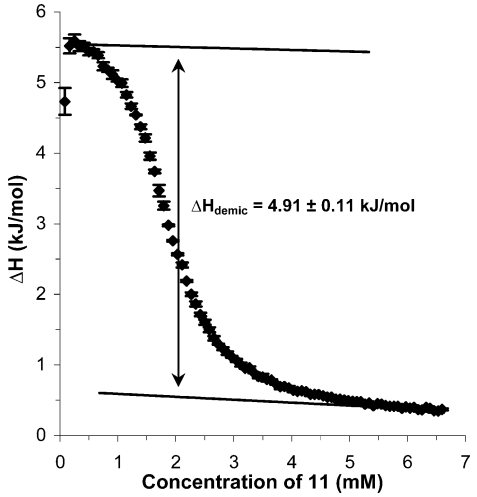


Figure 1. Molar reaction enthalpy as a function of the total concentration of **11** in the sample cell as observed during the titration of a micellar PBS solution of **11** (40 mM, 3 μL steps) into 1.4 mL of PBS at 37 °C. Values are the mean ± SEM of three replicate titrations. The enthalpy of demicellization is represented by the length of the arrow.

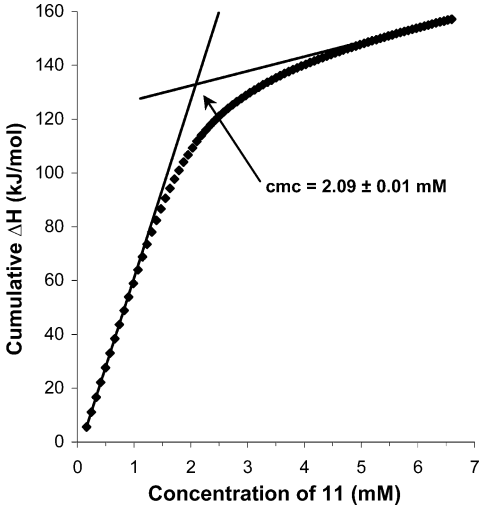


Figure 2. Cumulative molar reaction enthalpy as a function of the total concentration of **11** in the sample cell for the experiment presented in Figure 1. The cmc is defined as the concentration at which the two lines, fitted by linear regression to data well above and below the discontinuity in the curve, intersect. Values are the mean of three replicate titrations with percent relative SEM < 2%. The cmc is displayed as the mean ± SEM.

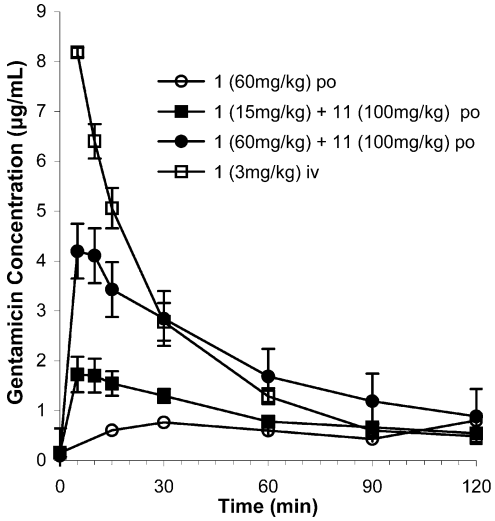


Figure 3. Gentamicin concentration in plasma as a function of time postadministration. Values are the mean ± SEM of four or five rats.

salts, for example, were effective as absorption enhancers only above their cmc.[29−31] The nonglycosylated bile salts showed correlation between cmc and effectiveness. In this study, the micellization of **11** was assessed by isothermal titration microcalorimetry.[32] Aliquots of a solution containing micelles of **11** in phosphate-buffered saline (PBS) at pH 7.4 were injected into a microcalorimeter sample cell filled with PBS solution, and the enthalpy change associated with each addition was measured. Figure 1 is a plot of reaction enthalpy (ΔH) as a function of the concentration of **11** in the sample cell. The shape of the plot is consistent with those obtained for other surfactants[33−36] and may be divided into two distinct regions within which the enthalpies are similar. For the first portion of the curve, the measured enthalpy changes are ascribed to the breaking up of micelles into monomers (demicellization). Once the concentration of **11** in the sample cell passes the cmc, the measured enthalpy changes represent the dilution enthalpy of the micelle.[33] The cmc is a concentration between these two distinct regions of the curve and can be estimated by various methods.[33,35,36] In this study the method of van Os was used in which the cumulative enthalpy was plotted as a function of surfactant concentration (Figure 2).[33] The plot showed a change of slope about the cmc, the value of which was calculated by selecting data well below and above the cmc, fitting these to a pair of straight lines by linear regression, and taking their point of intersection as the cmc. Using this process the cmc was estimated to be 2.09 ± 0.01 mM, a value similar to the excipients taurodeoxycholate (cmc = 1−2 mM),[29] a glycosylated cholic acid derivative (cmc = 1−2 mM),[29] and the semisynthetic saponin DS-1 (cmc = 1.09 mM).[11]

The enthalpy of demicellization (ΔH_{demic}), calculated as the enthalpy difference at the cmc between the two extrapolated lines in Figure 1, was 4.91 ± 0.11 kJ/mol.[33,36] This is in agreement with the results of Paula and co-workers, who examined the thermodynamics of micelle formation of octyl glucoside, sodium dodecyl sulfate (SDS), sodium cholate, and sodium deoxycholate, as a function of temperature.[36] They found that ΔH_{demic} increased with temperature and changed sign from negative to positive around room temperature. SDS, for example, exhibited ΔH_{demic} values −3.40 kJ/mol (10 °C), −1.40 kJ/mol (15 °C), 1.90 kJ/mol (25 °C), and 11.80 kJ/mol (50 °C).[36]

Gentamicin Absorption. The ability of **11** to enhance the gastrointestinal absorption of **1** was assessed in vivo using rats. The rat is an animal commonly employed in preclinical oral absorption studies, and rat gastrointestinal drug absorption correlates well with absorption in humans.[37−39] Compound **1** alone was administered intravenously (iv) and by oral gavage (po) or admixed with the liposaccharide excipient **11** and administered po. Mean plasma gentamicin concentrations as a function of time are shown in Figure 3 and pharmacokinetic parameters are detailed in Table 1. Coadministration of **1** (60 mg/kg) with **11** (100 mg/kg)

Table 1. Pharmacokinetic Parameters[a]

dose	C_{max}[b,c] (μg/mL)	t_{max}[d] (min)	AUC_{120}[c,e] (μg min/mL)	F[c,f] (%)
1 (60 mg/kg) po (control)	0.77 ± 0.05	30	70.5 ± 7.9	1.4 ± 0.3
1 (60 mg/kg) + **11** (100 mg/kg) po	4.20 ± 0.40[g]	5	240.0 ± 51.3[g]	4.9 ± 1.0[h]
1 (15 mg/kg) + **11** (100 mg/kg) po	1.73 ± 0.36	5	113.8 ± 14.1	9.2 ± 2.1[i,j]
1 (3 mg/kg) iv	8.19 ± 0.35	–	251.0 ± 25.7	100[k]

[a] n = 4−5 rats per group. [b] Maximum plasma gentamicin concentration. [c] Values are mean ± SEM. [d] Time to reach C_{max}. [e] Area under the curve. [f] Percent absolute bioavailability. [g] P < 0.001 cf. control. [h] P = 0.048 cf. control. [i] P = 0.034 cf. control. [j] Relative bioavailability cf. [**1** (60 mg/kg) + **11** (100 mg/kg) po] (f_{rel}) = 1.90 ± 0.44. [k] By definition.

Table 2. Gentamicin:Liposaccharide Molar and Charge Ratios

dose	molar ratio[a]	charge ratio[b]
1 (60 mg/kg) + **11** (100 mg/kg) po	1:2.7	1:0.54
1 (15 mg/kg) + **11** (100 mg/kg) po	1:11	1:2.2

[a] Calculated assuming gentamicin sulfate average FW = 710.0 g/mol. [b] **1** contains five basic nitrogens and **11** contains one acid group.

afforded a percent absolute bioavailability (F) of 4.9%, which represents a statistically significant (P = 0.048) 3-fold increase in bioavailability compared with control **1** (60 mg/kg) alone (F = 1.4%). Statistically significant (P < 0.001) improvements in C_{max} and AUC_{120} were also indicated by this mixture compared to control. Reduction of the dose of **1** to 15 mg/kg while the dose of **11** was maintained at 100 mg/kg produced a significant (P = 0.034) 6-fold increase in bioavailability to 9.2% when compared to the control.

The bioavailability of [**1** (15 mg/kg) + **11** (100 mg/kg) po] relative to [**1** (60 mg/kg) + **11** (100 mg/kg) po] (f_{rel}) was 1.90 ± 0.44. This provides a measure of relative performance of the two formulations. Typically two formulations are considered bioequivalent should f_{rel} be in the range 0.8−1.25.[40] In this case the two formulations are not equivalent, a finding that may be ascribed to the differences in gentamicin/liposaccharide molar and charge ratios. Table 2 details the molar and charge ratios for the mixtures of **1** and **11** studied. Although the formulations contain the same dose of liposaccharide (**11**), the formulation with the greater molar excess of **11** performed better. Given that ion-pairing between **11** and **1** may aid absorption, it should be noted that only the formulation [**1** (15 mg/kg) + **11** (100 mg/kg) po] contained sufficient liposaccharide (**11**) to completely neutralize **1** on a charge basis. Further investigation is warranted to determine the optimum gentamicin/liposaccharide ratio for oral absorption.

Figure 3 shows that when formulated with **11**, absorption of **1** was rapid with an observed t_{max} of 5 min. Such rapid absorption into the systemic circulation could be explained by absorption occurring at the level of the stomach. Alternatively, absorption may be intestinal, should the liquid formulation pass quickly from the stomach to the intestine. It has been shown both in fasted and fed animals that a substantial amount of a liquid dose enters the small intestine within one minute of gavage and results in the substances being absorbed rapidly.[41,42] While the highest C_{max} achieved in this study (4.20 μg/mL) was a significant (P < 0.001) 5.5 times greater than the control gentamicin C_{max} (0.77 μg/mL), it is marginally below the therapeutic range of 5−10 μg/mL.[43] It should be noted, however, that there exists substantial possibility of improvement in the delivery system by refining both the formulation of liposaccharide with gentamicin (e.g. dose, drug/liposaccharide ratio) and in the design of the liposaccharide excipient itself. The potential to modulate the physicochemical properties of the system by altering the length of the Laa alkyl chain and the nature of the sugar moiety[23−25] makes this a highly adaptable synthetic drug delivery system in comparison to methods utilizing natural or semisynthetic agents.[11,12,14,15,29]

Histopathology. Many penetration enhancers act by disrupting membrane integrity.[3] Sometimes the mechanism of enhanced absorption can be attributed to damage of the gastrointestinal mucosa.[3,44] For instance, the nonsurfactant sodium 5-methoxysalicylate caused mucosal damage to rat duodenum.[45,46] The surfactants SDS and SDC caused alterations to rat rectal mucosa.[47] Anions such as chloride and bromide, used to enhance absorption of quaternary ammonium drugs by ion-pairing, were shown to produce morphological changes in rat intestine.[48] In this study, the effect of **11** on the gastrointestinal mucosa was studied by light microscopy. The esophagi, stomach, and duodena were dissected from rats used in the pharmacokinetic study and fixed in formalin solution. After embedding, sectioning, and staining, the samples were examined for morphological changes. No morphological changes were found in either the esophagi or duodena. One rat from the treatment group [**1** (60 mg/kg) + **11** (100 mg/kg) po] exhibited slight gastric erosion, which could be attributed to **11**. The absence of major histological damage is a promising result, considering the deleterious effects typical of penetration enhancers.[3,44]

Conclusion. We have synthesized an anionic liposaccharide surfactant (**11**) for use as a drug delivery agent to improve the gastrointestinal absorption of charged, hydrophilic drugs. The cmc, elucidated by isothermal titration microcalorimetry, was shown to be similar to conventional penetration enhancers. The capability of **11** as a drug delivery agent in vivo was assessed in rats using the hydrophilic, polycation gentamicin (**1**) as a model drug. Compound **1** admixed with **11** significantly improved oral absorption of **1** compared to the control. Excipients based on the structure of **11** are of interest, since they combine features of both surfactants and ion-pairing agents and are easily modified synthetically to modulate physicochemical properties.

Experimental Section

Synthesis. Commercial reagents were used without further purification. Flash column chromatography was performed using silica gel 60, 230−400 mesh ASTM (Scharlau, Barcelona, Spain). TLC was performed on silica gel 60 F_{254} aluminum sheets (Merck, Darmstadt, Germany), and compounds were visualized by either ninhydrin dip (0.1% ninhydrin in ethanol) or ceric sulfate dip (15% aqueous H_2SO_4 saturated with ceric sulfate). All TLC plates were developed by heating after treatment with developing agent. Melting points were measured with a capillary apparatus and are uncorrected. The mobile phase for mass spectrometry and HPLC was solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in 90% acetonitrile/10% water). Mass spectra were recorded on

a Perkin-Elmer Sciex API 3000 mass spectrometer operating in positive ion electrospray mode (ESI-MS) or an Applied Biosystems Qstar Pulsar electrospray qtof mass spectrometer operating in positive ion electrospray mode (HRMS), using 70% B at 0.3 mL/min. NMR spectra were recorded on either a Varian Gemini 300 instrument (^{1}H, 300 MHz; ^{13}C, 75 MHz) or a Bruker AM 500 instrument (^{1}H, 500 MHz; ^{13}C, 125 MHz). The following abbreviations were used to indicate the peak multiplicity: s = singlet; d = doublet, t = triplet; m = multiplet, br = broad. Proton signals were assigned using gradient COSY45 spectra recorded on the Bruker instrument. IR spectra were recorded on a Shimadzu FTIR-8201PC by KBr disk (2 mg of compound in 200 mg of KBr). The purity of **11** was determined by HPLC-MS using the Perkin-Elmer Sciex API 3000 mass spectrometer operating in positive ion electrospray mode linked to a HPLC system (Shimadzu SCL-10A VP Controller, two LC-10AT VP Pumps, DGU-12A Degasser and an Agilent 1100 Series Auto Injector) operating at a flow rate of 0.3 mL/min. The chromatography conditions were as follows: CN, Agilent Zorbax SB-CN column (5 μm), 2.1 × 50 mm, linear gradient of 10% B to 100% B over 10 min; ODS, Agilent Zorbax SB-C18 column (5 μm) 2.1 × 50 mm, linear gradient of 30% B to 100% B over 10 min. An aliquot (10 μL) of a solution of **11** (0.1 mg/mL in 1:1 A/B) was injected and the purity calculated as the percent area of integrated peaks in the total ion count chromatogram (TIC) (mass range 120−1000 by 0.1 amu). The reported peaks displayed mass spectra consistent with compound **11**, a mixture of diastereomers.

Acetic 1,2,3,4-Tetra-*O*-acetyl-β-D-glucopyranuronic Anhydride (3).[27] D-Glucuronic acid (6.0 g, 31 mmol) was suspended in acetic anhydride (85 mL) and stirred at 0 °C in a 250 mL round-bottom flask fitted with a condenser and a calcium chloride drying tube. Iodine (510 mg, 2.01 mmol) was added slowly and the mixture allowed to warm to room temperature overnight (ca. 18 h). The solvent was coevaporated with toluene to give a pale, brown solid which was washed with ether and collected in a Buchner funnel. Repeated ether trituration afforded the title compound (**3**) as a white solid (7.9 g, 71%): TLC R_f 0.65 (ethyl acetate, ceric ammonium sulfate dip); mp 132 °C; ESI-MS, *m*/*z* 427 $[M + Na]^+$, 422 $[M + NH_4]^+$, 345 $[M - OAc]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.83 (1H, d, $J_{1,2}$ 6.8, H-1), 5.38 (1H, t, *J* 9.0 H-4), 5.30 (1H, t, *J* 8.7, H-3), 5.12 (1H, t, *J* 7.4, H-2), 4.35 (1H, d, $J_{4,5}$ 9.0, H-5), 2.28 (3H, s, Ac), 2.13 (3H, s, Ac), 2.06, 2.04 (9H, 2s, 3Ac overlapping); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7, 169.2, 169.1, 168.5, 164.7, 162.5, 91.3, 72.9, 71.2, 70.0, 68.0, 21.9, 20.6, 20.4, 20.3; FT-IR 2955, 1828, 1766, 1427, 1373, 1211, 1088 cm^{-1}.

1,2,3,4-Tetra-*O*-acetyl-β-D-glucopyranuronic Acid (4). Method A.[26] D-Glucuronic acid (6.00 g, 31 mmol) was suspended in acetic anhydride (85 mL) and stirred at 0 °C. Iodine (427 mg, 1.68 mmol) was added slowly over 0.5 h and stirring was continued at 0 °C for 2 h then a further 1 h at 25 °C. The solution was cooled to 0 °C, dry methanol (30 mL, 740 mmol) was added dropwise, and then the solution was allowed to stir for 18 h at 25 °C. The solution was concentrated and taken up in DCM (100 mL), washed with sodium thiosulfate (1 M solution, 2 × 100 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo to afford a pale, brown gum (8.3 g). The crude product was taken up in a mixture of ether, hexane, and chloroform (1:1:1, 120 mL) and crystallized upon concentration of the mixture. The crystalline product was triturated with ether to afford the title compound (**4**) (6.17 g, 55%) as a white powder.

Method B.[27,28] Compound **3** (2.04 g, 5.05 mmol) was dissolved in a mixture of THF (40 mL) and water (10 mL) and stirred overnight (ca. 18 h). The solvent was removed in vacuo to afford peracetylated glucuronic acid as a white powder in quantitative yield: TLC R_f 0.41 (MeOH/DCM 1:3, ceric ammonium sulfate dip); mp 149 °C; ESI-MS, *m*/*z* 385 $[M + Na]^+$, 380 $[M + NH_4]^+$, 303 $[M - OAc]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.57 (1H, br s, COOH), 5.77 (1H, d, $J_{1,2}$ 7.6, H-1), 5.28 (2H, m, H-3 and H-4 overlapping), 5.10 (1H, t, *J* 8.0, H-2), 4.23 (1H, d, $J_{4,5}$ 9.0, H-5), 2.08, 2.01, 2.01, 2.00 (12H, 4s, 4Ac); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 169.8, 169.7, 169.3, 169.0, 91.2, 72.3, 71.8, 70.1, 68.6, 20.6, 20.4, 20.4, 20.4; FT-IR 2954, 1728, 1427, 1373, 1211, 1042 cm^{-1}.

2-(*tert*-Butoxycarbonylamino)-D,L-tetradecanoic Acid (5). Compound **5** was prepared by a slightly modified literature procedure.[23] Sodium (3.81 g, 166 mmol) was dissolved in ethanol (100 mL) under nitrogen and diethyl acetamido malonate (30.0 g, 138 mmol) was added, followed by 1-bromododecane (48.2 g, 193 mmol). The solution was refluxed overnight under an atmosphere of nitrogen. Upon cooling the mixture was poured onto crushed ice (600 mL) and the precipitated product collected, air-dried, and then refluxed overnight in a solution of concentrated HCl/DMF (9:1, 200 mL). Upon cooling, the product precipitated and was collected, washed with ice cold water, and then air-dried to afford 2-amino-D,L-tetradecanoic acid hydrochloride (37.6 g, 97%): ESI-MS, *m*/*z* 244 $[M + H]^+$. 2-Amino-D,L-tetradecanoic acid hydrochloride (35.2 g, 126 mmol) was suspended in a solution of *tert*-butyl alcohol/water (2:3, 750 mL) and the pH was adjusted to 13 with sodium hydroxide (5 M). Di-*tert*-butyl dicarbonate (41.2 g, 189 mmol) in *tert*-butyl alcohol (75 mL) was added and the pH of the reaction maintained at 13 during the first 3 h by addition of sodium hydroxide (5 M). The solution was then left to stir overnight. The mixture was diluted with water (300 mL), and solid citric acid was added to acidify the mixture to pH 3. The mixture was extracted with ethyl acetate (5 × 200 mL), and the combined extracts were dried ($MgSO_4$) and evaporated to yield a crude product which was recrystallized from warm acetonitrile to afford the title compound (**5**) (24.2 g, 56%): mp 55−56 °C, literature mp 62−64 °C;[23] ESI-MS, *m*/*z* 366 $[M + Na]^+$, 361 $[M + NH_4]^+$, 344 $[M + H]^+$, 288 $[M - t\text{-Bu}]^+$, 244 $[M - Boc]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (1H, br s, COOH), 5.03 (1H, d, *J* 8.1, CONH), 4.28 (1H, m, α-CH), 1.9−1.5 (2H, m, β-CH_2), 1.43 (9H, s, $C(CH_3)_3$), 1.24 (20H, m, $10CH_2$), 0.87 (3H, t, *J* 6.2, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.6, 155.6, 80.0, 53.4, 32.4, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 28.3, 25.3, 22.7, 14.1.

2-(*tert*-Butoxycarbonylamino)-D,L-tetradecanoic Acid Benzyl Ester (6). Carboxylic acid **5** (4.10 g, 11.9 mmol) was dissolved in methanol (300 mL). The solution was neutralized with a 20% solution of Cs_2CO_3 (ca. 30 mL, ca. 18 mmol Cs_2CO_3) and the solvent removed in vacuo. DMF (50 mL) was added and the solvent was removed in vacuo. Addition and evaporation of DMF (50 mL) was repeated to afford the solid cesium salt, which was dried under reduced pressure. The cesium salt was dissolved in DMF (150 mL) and benzyl chloride (1.66 g, 1.51 mL, 13.1 mmol) was added. The mixture was stirred at 70 °C for 4 h. The solvent was removed in vacuo and the crude product taken up in ethyl acetate (200 mL) and washed with 5% HCl (2 × 100 mL), saturated bicarbonate (2 × 100 mL), and brine (100 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated to afford the title compound (**6**) (3.88 g, 75%) as an oil: ESI-MS, *m*/*z* 566 $[M + Cs]^+$, 451 $[M + NH_4]^+$, 434 $[M + H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (5H, m, Ar−H), 5.16 (3H, m, Ar−CH_2 overlapping with CONH), 4.34 (1H, m, α-CH), 1.9−1.5 (2H, m, β-CH_2), 1.44 (9H, s, $C(CH_3)_3$), 1.26 (20H, m, $10CH_2$), 0.89 (3H, t, *J* 10.9, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.7, 155.3, 135.4, 128.4, 128.2, 128.1, 79.6, 66.7, 53.4, 32.5, 31.8, 29.5, 29.4, 29.3, 29.1, 28.2, 25.1, 22.6, 14.0; HRMS calcd for $[M + H]^+$ 434.3264, found 434.3270.

1-D,L-(Benzyloxycarbonyl)tridecylammonium Trifluoroacetate (7). Benzyl ester **6** (471 mg, 1.09 mmol) was dissolved in DCM/TFA (20 mL, 1:1) and stirred for 1 h. The solvent was removed in vacuo and the residue was dissolved in acetonitrile/water (1:1) and lyophilized to afford the title compound (**7**) (463 mg, 95%): ESI-MS, *m*/*z* 334 $[M + H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (5H, m, Ar−H), 5.24 (1H, d, *J* 12.1, Ar−CH), 5.13 (1H, d, *J* 12.1, Ar−CH), 4.01 (1H, m, α-CH), 1.91 (2H, m, β-CH_2), 1.23 (20H, m, $10CH_2$), 0.90 (3H, t, *J* 6.4, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.7, 134.5, 128.7, 128.6, 128.5, 68.1, 53.1, 31.9, 30.5, 29.6, 29.6, 29.5, 29.3, 29.1, 29.0, 24.6, 22.7, 14.1; HRMS calcd for $[M + H]^+$ 334.2740, found 334.2731.

***N*-[1-D,L-(Benzyloxycarbonyl)tridecyl]-1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronamide (8).** Carboxylic acid **4** (2.19 g, 6.04 mmol) was dissolved in THF (50 mL). DIEA (1.6 g, 2.1 mL, 12 mmol) and HBTU (2.50 g, 6.59 mmol) were added, and the mixture was stirred for 10 min at room temperature. A solution of **7** (2.46 g, 5.49 mmol) in THF (50 mL) was added and the mixture was stirred overnight (ca. 18 h). The solvent was removed in vacuo to afford an oil which was taken up in DCM (200 mL) and washed with 5% HCl (2 × 100 mL), saturated bicarbonate (2 × 100 mL), water (50 mL), and brine (50 mL). After drying ($MgSO_4$) the solvent was removed in vacuo to give an oil which was purified by silica flash column chromatography (ethyl acetate/hexane, 1:2) to afford the title compound (**8**) (2.81 g, 75%): mp 65−67 °C; TLC R_f 0.37 (ethyl acetate/hexane 1:2, ninhydrin dip); ESI-MS, *m*/*z* 700 $[M + Na]^+$, 695 $[M + NH_4]^+$, 678 $[M + H]^+$, 618 $[M - AcOH + H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (5H, m, Ar−H), 6.82 (1H, m, amide NH), 5.76 (1H, m, H-1), 5.33−5.07 (5H, m, overlapping Ar−CH_2, H-2, H-3, H-4), 4.55 (1H, m, α-CH), 4.09 (1H, m, H-5), 2.10, 2.02, 1.99 (12H, 3s, 4 Ac overlapping), 1.9−1.5 (2H, m, β-CH_2), 1.23 (20H, m, 10CH_2), 0.85 (3H, t, *J* 6.6, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7, 171.6, 169.7, 169.7, 169.3, 169.1, 169.1, 168.7, 168.5, 165.6, 165.4, 135.2, 135.1, 128.5, 128.3, 128.2, 128.1, 91.2, 91.2, 73.0, 72.8, 71.9, 71.9, 70.1, 68.9, 68.7, 67.0, 67.0, 51.9, 51.8, 32.2, 32.1, 31.8, 29.5, 29.3, 29.2, 29.0, 29.0, 24.9, 24.7, 22.6, 20.6, 20.5, 20.5, 20.4, 14.0; HRMS calcd for $[M + H]^+$ 678.3484, found 678.3506.

***N*-[1-D,L-Carboxytridecyl]-1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronamide (9).** Benzyl ester **8** (2.68 g, 3.96 mmol) was dissolved in THF (50 mL). Pd/C catalyst (0.5 g, 10% Pd on C) was added and the solution was stirred overnight (ca. 18 h) under an atmosphere of H_2. The mixture was filtered through Celite and the solvent was evaporated in vacuo to afford the title compound (**9**) (2.31 g, 99%) as an oil: TLC R_f 0.16 (ethyl acetate/hexane 4:1, ninhydrin dip); ESI-MS, *m*/*z* 588 $[M + H]^+$, 528 $[M - AcOH + H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.72 (1H, br s, COOH), 6.83 (1H, m, amide NH), 5.79 (1H, d, *J* 7.6, H-1), 5.32−5.11 (3H, m, overlapping H-2, H-3, H-4), 4.52 (1H, m, α-CH), 4.16 (1H, m, H-5), 2.13, 2.04, 2.00 (12H, 3s, 4 Ac overlapping), 1.9−1.5 (2H, m, β-CH_2), 1.24 (20H, m, 10CH_2), 0.86 (3H, t, *J* 6.2, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.6, 175.4, 169.9, 169.6, 169.5, 169.3, 169.2, 168.8, 168.7, 166.1, 91.3, 91.2, 72.9, 72.8, 72.0, 71.9, 70.2, 69.0, 68.8, 51.8, 51.8, 32.0, 31.8, 29.6, 29.3, 29.2, 25.1, 25.0, 22.6, 20.7, 20.5, 14.0; HRMS calcd for $[M + H]^+$ 588.3014, found 588.3026.

***N*-[1-D,L-Carboxytridecyl]-α,*β*-D-glucopyranuronamide (10).** Compound **9** (2.20 g, 3.7 mmol) was dissolved in methanol (50 mL). Sodium methoxide solution (50 mL, 0.2 M) was added and the solution was stirred at room temperature for 2 h. The mixture was neutralized with ion-exchange resin (Amberlite IR-120 (H+)) and filtered, and then the solvent was removed in vacuo. The product was lyophilized from acetonitrile/water (1:1) to afford the title compound (**10**) (1.49 g, 95%): ESI-MS, *m*/*z* 442 $[M + Na]^+$, 420 $[M + H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.1−7.5 (1H, m, amide NH), 4.4−2.9 (m), 1.9−1.5 (2H, m, β-CH_2), 1.23 (20H, m, 10CH_2), 0.85 (3H, t, *J* 6.3, CH_3); HRMS calcd for $[M + H]^+$ 420.2591, found 420.2592.

Sodium *N*-[1-D,L-Carboxytridecyl]-α,*β*-D-glucopyranuronamide (11). The free acid **10** (0.701 g, 1.67 mmol) was suspended in water (50 mL) and sodium bicarbonate (3.34 mL, 0.5 M solution, 1.67 mmol) was added. The mixture was sonicated until a clear solution was obtained. The reaction mixture was lyophilized to afford the title compound (**11**) (0.734 g) in quantitative yield. ESI-MS, *m*/*z* 442 $[M + Na]^+$, 420 $[M + H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.6 (1H, m, amide NH), 4.4−2.9 (m), 1.68 (1H, m, β-CH), 1.51 (1H, m, β-CH), 1.23 (20H, m, 10CH_2), 0.85 (3H, t, *J* 6.8, CH_3); HRMS calcd for $[M + H]^+$ 420.2591, found 420.2621; HPLC-MS, CN t_R = 5.82 min, ODS t_R = 5.25, 5.69 min (overlapping), 95%.

Critical Micelle Concentration. Microcalorimetric titrations were performed using a MicroCal VP-ITC titration calorimeter (MicroCal Inc., Northampton, MA) under isothermal conditions (37 °C). Phosphate-buffered saline (PBS) was prepared using PBS tablets (Amresco, Solon, OH) and contained 137 mM NaCl, 2 mM KCl, and 10 mM phosphate buffer at pH 7.4. Solutions were sonicated prior to use for at least 10 min. The reaction cell (1.4395 mL) was filled with PBS. Aliquots (87 × 3 μL) of a PBS solution of **11** (40 mM) were injected into the reaction cell (injection duration 6 s) at intervals of 6 min using a 300 μL injection/stirrer syringe rotating at 300 rpm. The software Origin (version 5.0, Microcal Inc) and Microsoft Excel 2002 (Microsoft Corp., WA) were used for data analysis. The cmc was calculated by the intersection of two lines fitted by linear regression to data well below and above the cmc in a plot of cumulative ΔH against the concentration of **11** (Figure 1).[33] ΔH_{demic} was calculated by the enthalpy difference at the cmc, between two lines fitted by linear regression to data well below and above the cmc in a plot of ΔH against concentration of **11** (Figure 2).[33,36] Cmc and ΔH_{demic} are reported as the mean ± SEM of experiments performed in triplicate.

Gentamicin Absorption. Gentamicin sulfate was obtained from Sigma Chemical Co. (St. Louis, MO). Zoletil-100 (tiletamine hydrochloride 100 mg/mL, zolazepam hydrochloride 100 mg/mL, Virbac Pty Ltd, Peakhurst, Australia), Ilium Xylazil-20 (xylazine hydrochloride 20 mg/mL, Troy Laboratories Pty Ltd, Smithfield, Australia), and heparinized saline (heparin 10 IU/mL, Astra Pharmaceuticals Pty Ltd, N.Ryde, Australia) were purchased from Provet Pty Ltd (Brisbane, Australia). Sprague−Dawley rats were obtained from the Herston Medical Research Centre, The University of Queensland (Brisbane, Australia). Cannulation was performed with polyethylene tubing (0.96 mm od × 0.58 mm id, Tyco Electronics, Castle Hill, Australia). Blood samples were collected into heparinized tubes (1.3 mL microtubes, Sarstedt, Numbrecht, Germany). All animal experiments were approved by the University of Queensland Animal Ethics Committee.

Cannulation of the Femoral Artery. Male Sprague−Dawley rats (300−350 g) were anaesthetized with Zoletil (60 mg/kg ip) and xylazine (12 mg/kg ip) to facilitate insertion of a permanent polyethylene cannula into the femoral artery. Following surgery, rats were allowed to recover overnight with free access to water but not food. A heparinized saline block was used in the cannula to prevent the development of blood clots in the cannula.

Pharmacokinetic Study. The day after surgery, rats fasted for 15 h were dosed with either **1** or a mixture of **1** and **11** (dissolved in 1 mL of 5% DMSO in water adjusted to pH 8 with sodium hydroxide or hydrochloric acid). The formulations were administered via oral gavage using a blunt-tipped feeding needle while the animal was under light anaesthesia (O_2/CO_2, 1:1). The doses studied were [**1** (60 mg/kg)], [**1** (15 mg/kg) admixed with **11** (100 mg/kg)], or [**1** (60 mg/kg) admixed with **11** (100 mg/kg)]. Following oral administration of the drug formulations, blood samples (0.5 mL) were collected via the implanted intra-arterial cannula at regular predetermined time points over a 2 h period (0, 5, 10, 15, 30, 60, 90, 120 min postdose). After collection of the final sample, the rats were promptly euthanized by cervical dislocation following light anaesthesia (O_2/CO_2, 1:1). The blood samples were centrifuged (20 000*g* for 4 min) and the plasma harvested and stored frozen (−70 °C) until analysis for **1**. The iv study was performed in an identical manner, except animals were dosed with **1** (3 mg/kg dissolved in 0.3 mL of heparinized saline) injected via the lateral tail vein.

Plasma Gentamicin Assay. Plasma samples were assayed for gentamicin on an immunoassay analyzer (Abbott AxSYM System, Abbott Laboratories, IL) using a commercially available fluorescence polarization immunoassay kit (Abbott AxSYM Gentamicin assay, Abbott Laboratories, IL). The limit of detection of the assay was 0.30 μg/mL.

Analysis of Pharmacokinetic Data. The maximum plasma gentamicin concentration (C_{max}) and time to reach C_{max} (t_{max}) are observed values taken directly from analytical data. The area under the plasma gentamicin concentration versus time curve (AUC_{120}) was estimated using the linear trapezoidal rule for the time period 0−120 min. Percent absolute bioavailability

(F) was calculated using the equation

$$F = \frac{(AUC_{po})(dose_{iv})}{(AUC_{iv})(dose_{po})} \times 100\%$$

where AUC = AUC_{120}. Relative bioavailability (f_{rel}) was calculated by

$$f_{rel} = \frac{(AUC_{\mathbf{1},15})(dose_{\mathbf{1},60})}{(AUC_{\mathbf{1},60})(dose_{\mathbf{1},15})}$$

where the subscript (**1**,15) refers to the group [**1** (15 mg/kg) + **11** (100 mg/kg) po] and the subscript (**1**,60) refers to the group [**1** (60 mg/kg) + **11** (100 mg/kg) po]. Values for C_{max}, AUC_{120}, F, and f_{rel} are mean ± SEM for four or five rats per group. Means of C_{max} and AUC_{120} for oral absorption data were compared by one-way analysis of variance (ANOVA) combined with the Tukey multiple comparison procedure. F values were compared to control F using a two-tailed unpaired t-test with unequal variance (Welch's approximate t). Gentamicin/liposaccharide molar ratio was calculated using a gentamicin sulfate average formula weight of 710.0 g/mol calculated by assuming the gentamicin complex contained 41% gentamicin C_1, 27% gentamicin C_2, and 32% gentamicin C_{1a} as the sulfate.[49] The charge ratio was calculated on the basis that **11** contains one acid group and **1** contains five basic nitrogens.

Histopathology. Esophagi, stomach, and duodena from animals used in the pharmacokinetic study (two animals from each treatment group) were dissected, fixed in formalin, embedded in paraffin wax, and sectioned. The sections were stained with hematoxylin and eosin and examined by light microscopy.

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Supporting Information Available: HPLC-MS chromatograms for compound **11** are available free of charge via the Internet at http://pubs.acs.org.

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