

Article

# Glycosides of Nadifloxacin—Synthesis and Antibacterial Activities against Methicillin-Resistant *Staphylococcus aureus*

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**Abstract:** The increase in the number of bacteria that are resistant to multiple antibiotics poses a serious clinical problem that threatens the health of humans worldwide. Nadifloxacin (**1**) is a highly potent antibacterial agent with broad-spectrum activity. However, its poor aqueous solubility has limited its use to topical applications. To increase its solubility, it was glycosylated herein to form a range of *trans*-linked (**3a–e**) and *cis*-linked (**7a,b**) glycosides, each of which was prepared and purified to afford single anomers. The seven glycoside derivatives (**3a–e**, **7a,b**) were examined for potency against eight strains of *S. aureus*, four of which were methicillin-resistant. Although less potent than free nadifloxacin (**1**), the  $\alpha$ -L-arabinofuranoside (**3a**) was effective against all strains that were tested (minimum inhibitory concentrations of 1–8  $\mu\text{g}/\text{mL}$  compared to 0.1–0.25  $\mu\text{g}/\text{mL}$  for nadifloxacin), demonstrating the potential of this glycoside as an antibacterial agent. Estimation of Log P as well as observations made during preparation of these compounds reveal that the solubilities of the glycosides were greatly improved compared with nadifloxacin (**1**), raising the prospect of its use in oral applications.

**Keywords:** *Staphylococcus aureus*; MRSA; nadifloxacin; glycoside

**Citation:** Hutchins, M.; Bovill, R.A.; Stephens, P.J.; Brazier, J.A.; Osborn, H.M.I. Glycosides of Nadifloxacin—Synthesis and Antibacterial Activities against Methicillin-Resistant *Staphylococcus aureus*. *Molecules* **2022**, *27*, 1504. <https://doi.org/10.3390/molecules27051504>

Academic Editor: Michio Kurosu

Received: 17 December 2021

Accepted: 16 February 2022

Published: 23 February 2022

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## 1. Introduction

Staphylococci are commonly found in the environment and are major colonizers of human skin [1,2]. *Staphylococcus aureus*, the major human pathogen of the genus, is mostly harmless, but in certain situations it can cause severe illness, such as endocarditis, pneumonia, sepsis and toxic shock syndrome [3,4]. Infections were initially treated with benzylpenicillin, but by the late 1950s, resistant strains of the bacteria that produced  $\beta$ -lactamase were increasingly being isolated. In 1959, the  $\beta$ -lactamase-resistant antibiotic methicillin was introduced, but since then there has been a steady increase in the prevalence of methicillin-resistant strains of bacteria, and last-resort drugs such as vancomycin are increasingly used to treat infections [5,6]. The isolation of strains with reduced susceptibility to this drug, termed vancomycin-intermediate-resistant *S. aureus* (VISA), has raised the prospect of there being no antibacterial therapy for such strains [7].

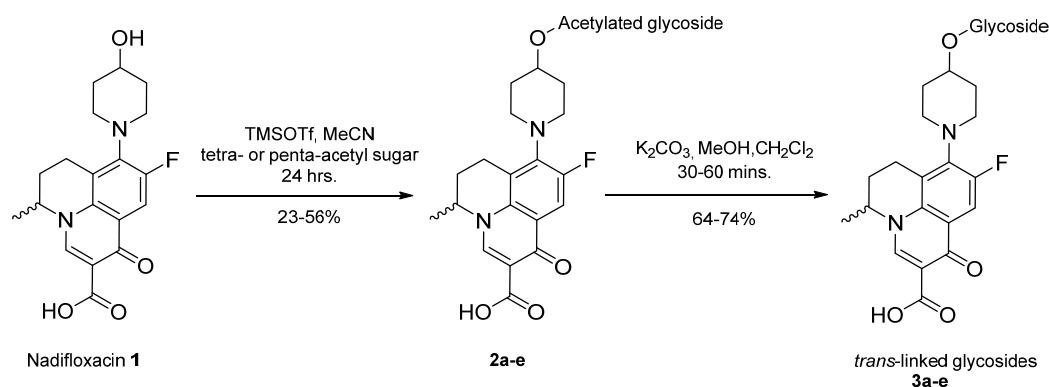
Nadifloxacin (**1**) (Scheme 1) is a broad-spectrum fluoroquinolone antibiotic that demonstrates high potency against aerobic Gram-positive and Gram-negative organisms and anaerobes. It has also been shown to be highly effective against methicillin-resistant *Staphylococcus aureus*, and low incidence of resistance is noted [8,9]. Unfortunately, nadifloxacin is poorly soluble in water and can only be used medicinally in topical ointments to treat acne and other skin infections [10,11]. Many attempts have been made to increase the aqueous solubility of nadifloxacin and thus enable it to be used in oral administration, for example by forming carboxylate salts, esters and peptides and by incorporating the drug in microemulsions and dendrimers [12–17]. However, the production of a suitable commercial derivative has so far been unsuccessful.

An alternative approach to improve the solubility of nadifloxacin is to glycosylate the free hydroxyl group on the piperidine ring (Scheme 1). Since this hydroxyl group is required

for nadifloxacin to have antibacterial properties, masking of this position by formation of a glycoside would serve a dual purpose. Firstly, it would increase the aqueous solubility of nadifloxacin as required. Secondly, it would afford glycosides that would be less toxic than nadifloxacin itself. Specific bacteria that express the corresponding glycosidase enzyme would, however, be able to hydrolyse the glycosides to afford nadifloxacin, potentially affording targeted treatments for specific bacterial infections [18–21]. This approach was explored herein by synthesising and testing seven different nadifloxacin glycosides (**3a–e**, **7a,b**) prepared from racemic nadifloxacin. The minimum inhibitory concentration (MIC) of each glycoside was then determined by means of a doubling dilution method.

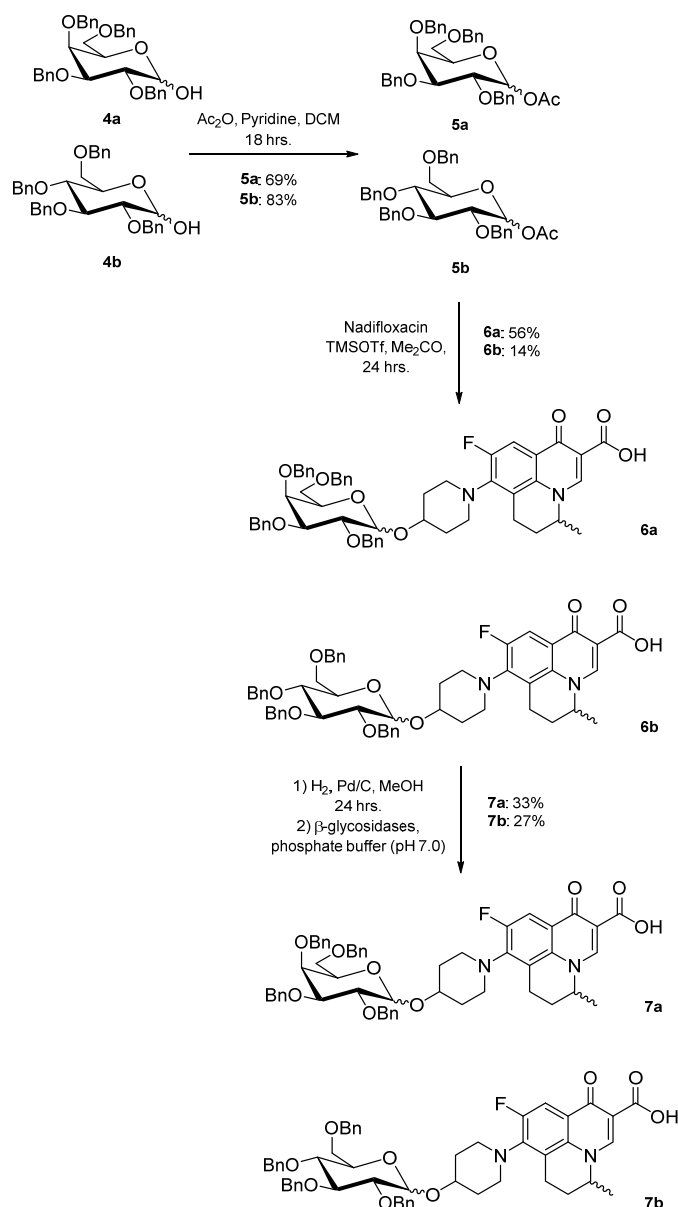
## 2. Results and Discussion

To prepare the different anomers of the nadifloxacin glycosides, two different synthetic approaches were used. The *trans*-linked nadifloxacin glycosides, specifically  $\alpha$ -L-arabinofuranoside (**3a**),  $\beta$ -D-galactopyranoside (**3b**),  $\beta$ -D-glucopyranoside (**3c**),  $\alpha$ -D-mannopyranoside (**3d**) and  $\beta$ -D-xylofuranoside (**3e**), were synthesised by reacting nadifloxacin (**1**) with the *O*-acetylated glycosides in the presence of 10 molar equivalents of trimethylsilyl triflate (TMSOTf) in anhydrous acetonitrile (Scheme 1). The glycosides (**2a–2e**) were deprotected by treatment with potassium carbonate in methanol and then purified by adjusting the pH of the crude deprotection mixture to about 3.0 with formic acid and eluting from a reverse-phase (C-18) flash chromatographic column with 0.1% formic acid/MeCN (7:3).



**Scheme 1.** Synthesis of *trans*-linked nadifloxacin glycosides, specifically  $\alpha$ -L-arabinofuranoside (**3a**),  $\beta$ -D-galactopyranoside (**3b**),  $\beta$ -D-glucopyranoside (**3c**),  $\alpha$ -D-mannopyranoside (**3d**) and  $\beta$ -D-xylofuranoside (**3e**), from the respective protected intermediates (**2a–e**).

The *cis*-galactoside (**7a**) and *cis*-glucoside (**7b**) were obtained by changing the reaction solvent and glycoside-protecting groups to favour the formation of the thermodynamic product (Scheme 2). The tetra-*O*-benzyl glycosides (**4a**) and (**4b**) were acetylated with acetic anhydride, then coupled with nadifloxacin (**1**) using TMSOTf in anhydrous acetone. This afforded a mixture of glycoside anomers with  $\alpha$ : $\beta$  ratios of 1:1.59 for the galactoside (**6a**) and 1:1.23 for the glucoside (**6b**), as evidenced by <sup>1</sup>H Nuclear Magnetic Resonance (NMR) spectroscopic analysis. The benzyl ethers were then removed by hydrogenolysis over palladium on charcoal to afford (**7a**) and (**7b**) as a mixture of anomers. These anomeric mixtures could not be separated by chromatography, and instead the  $\beta$ -glycoside was selectively hydrolysed using the corresponding  $\beta$ -glycosidase enzymes— $\beta$ -galactosidase from *Escherichia coli* in phosphate-buffered saline pH 7.0 or  $\beta$ -glucosidase from almonds, in acetate buffer, pH 6.0. The mixtures were then purified using flash chromatography as described above. All intermediates (**2a–e**), (**5a–b**), (**6a–b**) and deprotected glycosides (**3a–e**) and (**7a,b**) were characterised by <sup>1</sup>H and <sup>13</sup>C NMR and IR, spectroscopic analysis and mass spectrometric analysis. Purity was determined by High Performance Liquid Chromatography (HPLC) to be > 97% prior to subsequent analysis of the inhibitory properties of the glycosides (**3a–e**) and (**7a,b**).



**Scheme 2.** Synthesis and resolution of nadifloxacin- $\alpha$ -galactoside (**7a**) and  $\alpha$ -glucoside (**7b**).

All of the synthesised nadifloxacin glycosides (**3a–e**) and (**7a,b**) were soluble in water at physiological pH as demonstrated in the final enzymatic resolution step in Scheme 2, wherein the product was dissolved in aqueous systems to allow for enzymatic hydrolysis. The octanol–water coefficients (Log P) were also calculated as 0.05 for the hexose glycosides and 0.51 for the pentoses, compared to 1.79 for underivatized nadifloxacin (**1**) (calculated using ChemDraw 12.0, Perkin-Elmer, Cambridge, UK).

It should be noted that racemic nadifloxacin was used in all glycoside syntheses, and the products (**3a–e**) and (**7a,b**) were isolated as mixtures of diastereomers. This afforded more complex NMR spectra than expected with some peaks unresolved and thus quoted as multiplets.

Since nadifloxacin (**1**) was developed as a potent antimicrobial for the treatment of MRSA, the antimicrobial activities of the synthesised nadifloxacin glycosides were determined for a range of *Staphylococcus aureus* strains. The lowest concentration at which bacterial growth could not be detected was recorded as the minimum inhibitory concentration (MIC). All of the *Staphylococcus aureus* strains were found to be sensitive to nadifloxacin, and all of the strains were found to be sensitive to, or intermediately sensitive to, the

glycosides. However, all of the glycosides (**3a–e**) and (**7a,b**) had MICs that were higher than free nadifloxacin (**1**) (Table 1), presumably due to the requirement of hydrolysis to release the free nadifloxacin. This could indicate that the nadifloxacin glycosides do not induce the genes that produce the proteins required for transport and hydrolysis of the glycosides. There was variation in the MICs between glycosides and between strains for the same glycoside, probably due to differences in the rate of uptake of the glycoside by the bacteria and the amount of glycosidase that was expressed by the bacteria. Free nadifloxacin (**1**) was slightly more inhibitory for the MRSA strains than for non-MRSA organisms (0.125 µg/mL compared to 0.25–0.5 µg/mL), but there was no general pattern to the resistance of the glycosides. Of the glycosides, the α-L-arabinofuranoside (**3a**) was the most potent with MICs of 1–8 µg/mL for all of the strains that were examined.

**Table 1.** MICs (µg/mL) for nadifloxacin (**1**) and synthesised glycosides (**3a–e**) and (**7a,b**) against drug-susceptible and -resistant *S. aureus* in TSA.

Species	Strain	1	3a	3b	3c	3d	3e	7a	7b
<i>S. aureus</i>	ATCC 6538	0.25	2	8	64	16	2	8	16
	ATCC 6538p	0.25	2	8	128	16	1	64	16
	ATCC 9144	0.5	1	8	32	8	1	4	16
	ATCC 25923	0.25	2	8	64	16	2	8	32
<i>S. aureus</i> (MRSA)	ATCC 33591	0.125	1	32	64	128	1	2	4
	ATCC 43300	0.125	2	2	4	8	32	64	128
	NCTC 10442	0.125	8	2	2	4	32	2	128
	NCTC 12493	0.125	2	32	8	64	32	32	32

**1:** nadifloxacin, **3a:** α-arabinoside, **3b:** β-galactoside, **3c:** β-glucoside, **3d:** α-mannoside, **3e:** β-xyloside, **7a:** α-galactoside, **7b:** α-glucoside.

### 3. Materials and Methods

NMR spectra were recorded on a Bruker DPX spectrometer (400 MHz), and chemical shifts are quoted in ppm relative to tetramethylsilane as internal standard using the following abbreviations: s, singlet, d, doublet, at, apparent triplet, as, apparent singlet and m, multiplet. Liquid Chromatography Mass Spectrometry (LCMS) was accomplished using a ThermoFisher Scientific Accela LC system coupled to a ThermoFisher Scientific LTQ Fleet Ion Trap Mass Spectrometer (ThermoFisher Scientific, Loughborough, UK). Melting points were recorded on a TA Instruments DSC Q2000 instrument heating at 10 °C/min (Hertfordshire, UK). FTIR spectra were recorded on a ThermoFisher Scientific Nicolet iS10 instrument. Thin-layer chromatography was performed using ALUGRAM SIL G pre-coated plates (Macherey-Nagel, Germany). The purity of deprotected samples was achieved using an Agilent 1100 series HPLC with a ThermoFisher Scientific Hypersil Gold Column (50 × 4.6 mm, 3 µm) eluting 0.1% (v/v) formic acid in water/MeCN (85:15), 2 mL/min, UV absorbance at 254 nm.

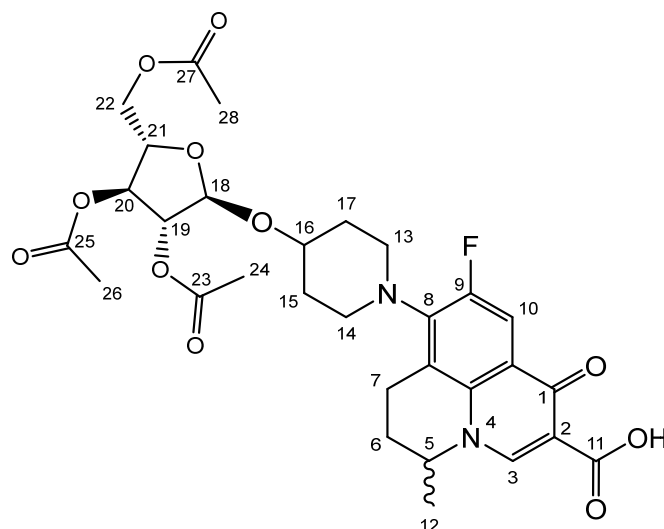
All culture media was “Oxoid” from ThermoFisher Scientific, Basingstoke, UK. Nadifloxacin was purchased from AOKChem (Shanghai, China) as a racemate and was used without further purification. Per-*O*-acetylated and 2,3,4,6-tetra-*O*-benzyl glucose and galactose were purchased from Carbosynth (Berkshire, UK). All other reagents were purchased from Sigma-Aldrich (Poole, Dorset, UK) or Fisher Scientific (Loughborough, UK).

#### Synthesis of 1,2-*trans* glycosides promoted by 10 molar equivalents of TMSOTf—General method.

Nadifloxacin (**1**) (5 g, 13.9 mmol) was suspended in anhydrous acetonitrile (250 mL), and to this TMSOTf (2.51 mL, 13.9 mmol) was added, and the solution was stirred. Following this were the additions of the per-*O*-acetylated glycoside (either 1,2,3,5-tri-*O*-acetyl-α-L-arabinofuranoside, 1,2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside, 1,2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside, 1,2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside or 1,2,3,5-tri-*O*-acetyl-β-D-xylofuranoside, 13.9 mmol) followed by TMSOTf (22.59 mL, 124.9 mmol). Reactions were stirred at room temperature under an atmosphere of nitrogen for 24 h. The

reaction mixtures were then diluted with anhydrous dichloromethane (250 mL), washed with sat.  $\text{NaHCO}_3$  ( $2 \times 100$  mL) and brine ( $2 \times 100$  mL), dried over  $\text{MgSO}_4$  and concentrated in vacuo. The products were purified on silica columns and eluted with toluene (A) and acetonitrile (B) at 14% B to 36% B over 5 column volumes and maintained at 36% for 10-column volumes.

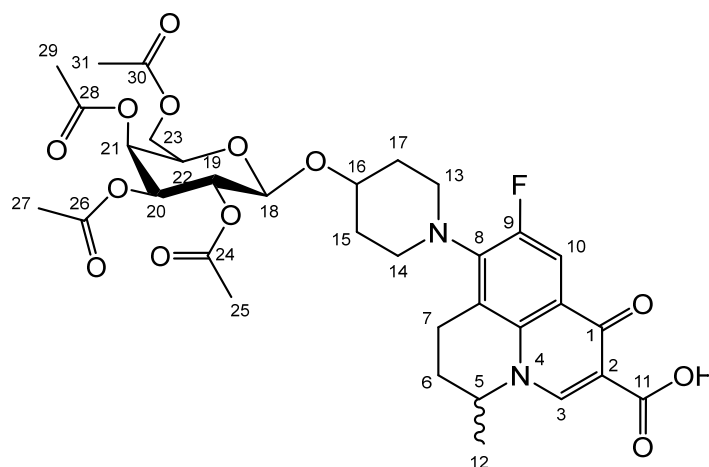
#### Nadifloxacin 2,3,5-tri-*O*-acetyl- $\alpha$ -L-arabinofuranoside (2a)



Nadifloxacin (1) (5 g, 13.9 mmol) was added to anhydrous acetonitrile and reacted with 1 molar equivalent of TMSOTf, 1,2,3,5-tetra-*O*-acetyl- $\alpha$ -L-arabinofuranoside (4.53, 13.9 mmol), followed by 9 molar equivalents of TMSOTf overnight to yield the product (2a) as a white powder.

Yield (4.81 g, 56%), m.p. 93–106 °C,  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.69 (1H, s, C3-H), 7.98 (1H, d,  $J = 12.5$  Hz, C10-H), 5.25–5.21 (1H, m, C18-H), 5.13–5.08 (1H, m, C21-H), 5.02 (1H, d,  $J = 5.0$  Hz, C19-H), 4.56 (1H, dd,  $J = 7.0, 3.5$  Hz, C5-H), 4.44 (1H, dt,  $J = 11.5, 2.5$  Hz, C22-Ha), 4.34–4.28 (1H, m, C20-H), 4.28–4.21 (1H, m, C22-Hb), 3.31 (1H, m, C7-Ha), 2.95–2.79 (1H, m, C7-Hb), 2.22–2.18 (2H, m, C6-H<sub>2</sub>), 2.16–2.09 (9H, m, acetyl CH<sub>3</sub>), 2.07–1.63 (4H, m, C15-H<sub>2</sub> and C17-H<sub>2</sub>), 1.52 (3H, d,  $J = 6.5$  Hz).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 206.98 ketone (C=O), 170.62, 170.19, 169.84 (acetyl C=O), 167.26 (carbonyl C=O), 147.35 (C3), 137.83, 133.35 (aromatic C), 110.66 (C10), 107.63 (C18), 81.71 (C21), 80.32 (C20), 76.80 (C19), 63.29 (C22), 57.94 (C5), 32.71 (C15, C17), 30.91 (C16), 25.97 (C6), 21.44, 20.78, 20.75 (acetyl CH<sub>3</sub>), 20.20 (C5-CH<sub>3</sub>), 18.83 (C7). FT-IR  $\nu$   $\text{cm}^{-1}$ : 2936.38 (w, carboxyl OH), 1738.96 (carboxylic or ester C=O), 1621.49 (aromatic C=C). MS +ESI found 619.2125 (MH<sup>+</sup>) C<sub>30</sub>H<sub>36</sub>FN<sub>2</sub>O<sub>11</sub> required 619.2298.

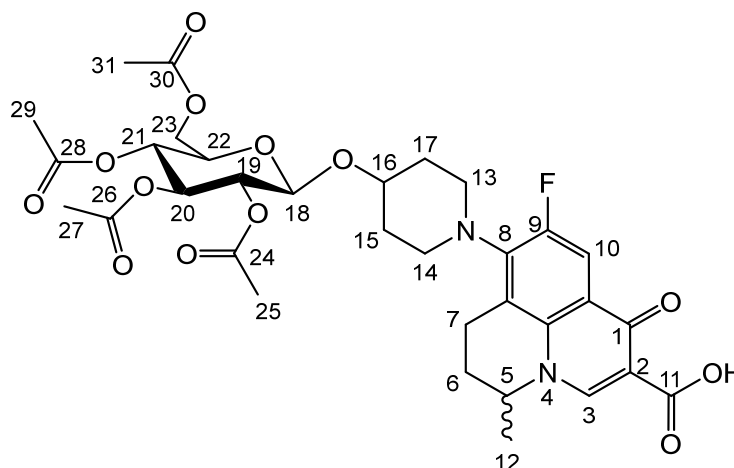
#### Nadifloxacin 2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-galactopyranoside (2b)



Nadifloxacin (**1**) (5 g, 13.9 mmol) was added to anhydrous acetonitrile and reacted with 1 molar equivalent of TMSOTf, 1,2,3,4,6-penta-*O*-acetyl- $\beta$ -D-galactopyranoside (5.6 g, 13.9 mmol) followed by 9 molar equivalents of TMSOTf overnight to yield the product (**2b**) as a white powder.

Yield (4.98 g, 52%). m.p. 151–165 °C,  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.63 (1H, s, C3-H), 7.92 (1H, d,  $J = 12.0$  Hz, C10-H), 5.39–5.31 (1H, m, C21-H), 5.19 (1H, at,  $J = 9.0$  Hz, C19-H), 5.02–4.96 (1H, m, C20-H), 4.57 (1H, d,  $J = 8.0$  Hz, C18-H), 4.53–4.45 (1H, m, C5-H), 4.15 (1H, dd,  $J = 11.5, 7.0$  Hz, C23-Ha), 4.06 (1H, dd,  $J = 11.5, 7.0$  Hz, C23-Hb), 3.87 (1H, at,  $J = 7.0$  Hz, C22-H), 3.30–2.90 (5 H, m, C7-Ha, C13-H<sub>2</sub>, C14-H<sub>2</sub>), 2.79 (1 H, dt,  $J = 18.0, 8.5$  Hz, C7-Hb), 2.14 (2H, m, C6-H<sub>2</sub>), 2.04 (2H, s, acetyl CH<sub>3</sub>), 2.01 (2H, s, acetyl CH<sub>3</sub>), 1.99 (3 H, s, acetyl CH<sub>3</sub>), 1.94 (3 H, s, acetyl CH<sub>3</sub>), 1.89–1.55 (4H, m, C15-H<sub>2</sub>, C17-H<sub>2</sub>), 1.46 (3H, d,  $J = 6.5$  Hz, C12-H<sub>3</sub>).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 170.41, 170.28, 170.19, 167.25 (carboxyl C=O), 146.35 (C3), 133.61 (aromatic C), 110.81, 110.59 (C10), 100.33 (C18), 70.86 (C20), 70.66 (C22), 68.98 (C19), 66.99 (C21), 61.23 (C23), 57.94 (C5), 25.97 (C6), 20.81, 20.71, 20.62 (Acetyl CH<sub>3</sub>), 20.23 (C12). FT-IR  $\nu$   $\text{cm}^{-1}$ : 2976.02 (w, carboxyl OH), 1748.35 (s, ketone C=O), 1731.64 (carboxylic or ester C=O), 1624.15 (aromatic C=C). MS +ESI found 691.2141 ( $\text{MH}^+$ )  $\text{C}_{33}\text{H}_{40}\text{FN}_2\text{O}_{13}$  required 691.2509.

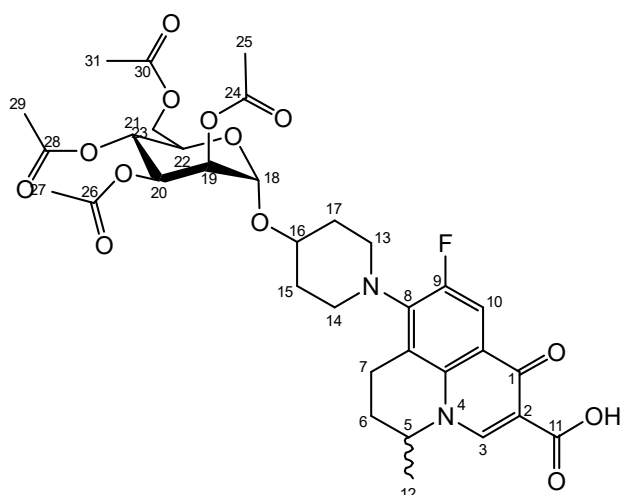
#### Nadifloxacin 2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranoside (**2c**)



Nadifloxacin (**1**) (5 g, 13.9 mmol) was added to anhydrous acetonitrile and reacted with 1 molar equivalent of TMSOTf, 1,2,3,4,6-penta-*O*-acetyl- $\beta$ -D-glucopyranoside (5.6 g, 13.9 mmol) followed by 9 molar equivalents of TMSOTf overnight to yield the product (**2c**) as a white powder.

Yield (2.73 g, 29%). m.p. 156–159 °C,  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.68 (1H, s, C3-H), 7.96 (1H, d,  $J = 12.0$  Hz, C10-H), 5.22 (1H, dd,  $J = 9.5, 2.0$  Hz, C20-H), 5.08 (1H, dd,  $J = 9.5, 2.0$  Hz, C21-H), 5.04–4.96 (1H, m, C19-H), 4.67 (1H, d,  $J = 8.0$  Hz, C18-H), 4.57 (1H, dt,  $J = 7.0, 3.5$  Hz, C5-H), 4.25 (1H, dd,  $J = 12.5, 5.0$  Hz, C23-Ha), 4.17–4.10 (1H, m, C23-Hb), 3.72 (1H, ddd,  $J = 10.0, 4.5, 2.5$  Hz, C22-H), 3.33–2.94 (5 H, m, C7-Ha, C13-H<sub>2</sub>, C14-H<sub>2</sub>), 2.84 (1H, ddt,  $J = 17.0, 10.5, 5.5$  Hz, C7-Hb), 2.15 (2H, m, C6-H<sub>2</sub>), 2.07 (3H, acetyl CH<sub>3</sub>), 2.04 (3H, acetyl CH<sub>3</sub>), 2.02 (3H, acetyl CH<sub>3</sub>), 2.00 (3H, acetyl CH<sub>3</sub>), 1.50 (3H, d,  $J = 6.5$  Hz, C12-H<sub>3</sub>).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 207.66 (C1), 170.78, 170.38, 169.51, 167.64 (carboxyl C=O), 146.45 (C3), 133.64 (aromatic C), 107.39 (C10), 99.17 (C18), 72.76 (C20), 71.71 (C22), 71.44 (C19), 68.43 (C21), 61.98 (C23), 57.99 (C5), 30.89 (C6), 20.1 (acetyl CH<sub>3</sub>), 20.14 (C12). FT-IR  $\nu$   $\text{cm}^{-1}$ : 2973.23 (w, carboxyl OH), 1748.42 (s, ketone C=O), 1725.81 (carboxylic or ester C=O), 1672.21 (aromatic C=C). MS +ESI found 691.2930 ( $\text{MH}^+$ )  $\text{C}_{33}\text{H}_{40}\text{FN}_2\text{O}_{13}$  required 691.2509.

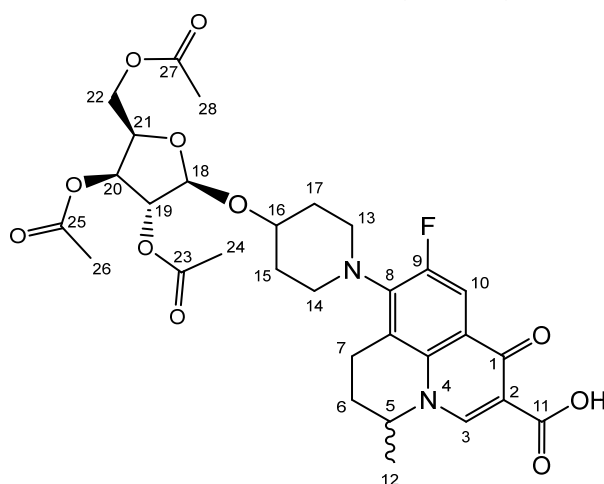
#### Nadifloxacin 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-mannopyranoside (**2d**)



Nadifloxacin (**1**) (5 g, 13.9 mmol) was added to anhydrous acetonitrile and reacted with 1 molar equivalent of TMSOTf, 1,2,3,4,6-penta-*O*-acetyl-*D*-mannopyranoside (5.6 g, 13.9 mmol) followed by 9 molar equivalents of TMSOTf overnight to yield the product (**2d**) as a white powder.

Yield (2.20 g, 23%). m.p. 190–204 °C  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.63 (1H, s, C3-H), 7.92 (1H, d,  $J = 12.0$  Hz, C10-H), 5.34 (1H, dd,  $J = 10.5, 3.0$  Hz, C20-H), 5.24 (1H, dd,  $J = 10.0, 3.0$  Hz, C21-H), 5.20–5.14 (1H, m, C19-H), 4.99–4.95 (1H, m, C18-H), 4.55–4.44 (1H, m, C5-H), 4.23 (1H, dd,  $J = 12.0, 4.5$  Hz, C23-Ha), 4.12–4.01 (2H, m, C23-Hb, C22-H), 3.50–2.93 (3H, m, C7-Ha, C13-H<sub>2</sub>, C14-H<sub>2</sub>), 2.81 (1H, dt,  $J = 17.5, 9.5$  Hz, C7-Hb), 2.11 (5H, m, C6-H<sub>2</sub>, acetyl CH<sub>3</sub>), 2.04 (acetyl CH<sub>3</sub>), 2.00 (acetyl CH<sub>3</sub>), 1.95 (acetyl CH<sub>3</sub>), 1.91–1.52 (4H, m, C15-H<sub>2</sub>, C17-H<sub>2</sub>) 1.47 (3H, d,  $J = 6.5$  Hz, C12-H<sub>3</sub>).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 177.36 (C11), 170.59, 170.22, 170.01, 169.72 (carboxyl C=O), 146.39 (C3), 133.65 (aromatic C), 110.79, 110.56 (C10), 107.66 (aromatic C), 95.97 (C18), 70.10 (C19), 69.01 (C20), 68.77 (C22), 66.31 (C21), 62.56 (C23), 57.96 (C5), 25.95 (C6), 20.95, 20.75, 20.73 (acetyl CH<sub>3</sub>), 20.24 (C12), 18.84 (C7). FT-IR  $\nu$   $\text{cm}^{-1}$ : 2956.60 (w, carboxyl OH), 1737.23 (carboxylic or ester C=O), 1617.60 (aromatic C=C). MS +ESI found 691.2372 ( $\text{MH}^+$ )  $\text{C}_{33}\text{H}_{40}\text{FN}_2\text{O}_{13}$  required 691.2509.

#### Nadifloxacin 2,3,5-tri-*O*-acetyl- $\beta$ -*D*-xylofuranoside (**2e**)



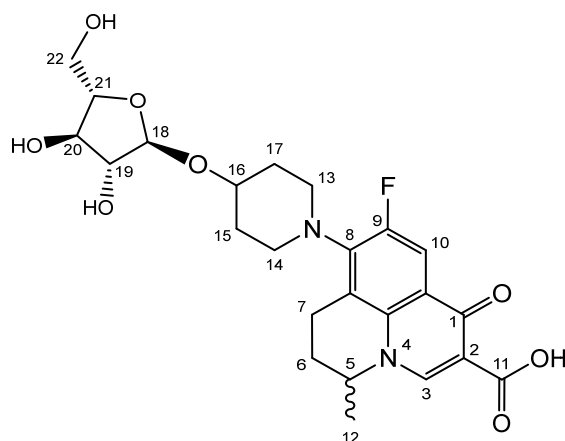
Nadifloxacin (**1**) (5 g, 13.9 mmol) was added to anhydrous acetonitrile and reacted with 1 molar equivalent of TMSOTf, 1,2,3,5-tetra-*O*-acetyl- $\beta$ -*D*-xylofuranoside (4.53 g, 13.9 mmol) followed by 9 molar equivalents of TMSOTf overnight to yield the product (**2e**) as a white powder.

Yield (4.72 g, 55%). m.p. 84–95 °C,  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.70 (1H, s, C3-H), 7.98 (1H, d,  $J = 12.5$  Hz, C10-H), 5.34 (1 H, dd,  $J = 6.0, 2.0$  Hz, C20), 5.22–5.19 (1H, m, C18-H), 5.15–5.12 (1H, m, C19-H), 4.65–4.50 (2H, m, C5-H, C21-H), 4.37–4.11 (2H, m, C22-H<sub>2</sub>), 3.37–3.01 (5H, m, C7-Ha, C13-H<sub>2</sub>, C14-H<sub>2</sub>), 2.90 (1H, ddd,  $J = 18.0, 11.5, 7.0$  Hz, C7-Hb), 2.23–1.69 (m, C6, acetyl CH<sub>3</sub>, C15-H<sub>2</sub>, C17-H<sub>2</sub>), 1.52 (3H, d,  $J = 7.0$  Hz).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 177.85 (C11), 170.78, 170.14, 169.94, 167.32 (acetyl C=O), 146.76 (C3), 138.33, 134.19 (aromatic C), 110.60, 110.36 (C10), 104.69 (C18), 81.38 (C19), 78.47 (C21), 75.27 (C20), 63.44 (C22), 58.49 (C5), 26.24 (C6), 21.00, 20.86, (acetyl C), 20.28 (C12), 19.25 (C13 or C14). FT-IR  $\nu$   $\text{cm}^{-1}$ : 2936.38 (w, carboxyl OH), 1738.96 (carboxylic or ester C=O), 1621.49 (aromatic C=C). MS +ESI found 619.2062 ( $\text{MH}^+$ )  $\text{C}_{30}\text{H}_{36}\text{FN}_2\text{O}_{11}$  required 619.2298.

#### Deprotection of per-O-acetylated nadifloxacin glycosides—General method

The isolated glycosides (**2a–e**) were de-O-acetylated using a mixture of anhydrous dichloromethane and methanol (1:6) and potassium carbonate (50% *w/w*) over 30–60 min. The mixtures were adjusted to approximately pH 3 by the addition of formic acid and were purified on C18 columns and eluted with 0.1% (*v/v*) formic acid in water (A) and acetonitrile (B) isocratically at 7:3.

#### Nadifloxacin $\alpha$ -L-arabinofuranoside (**3a**)

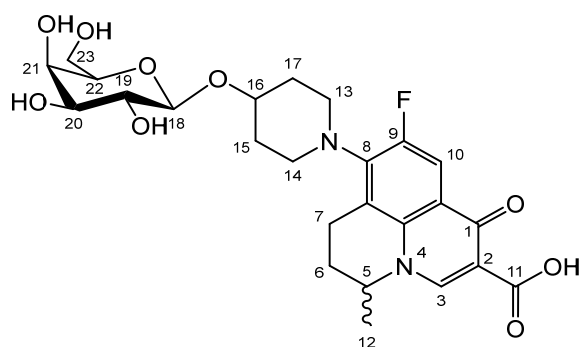


Nadifloxacin 2,3,5-tri-O-acetyl- $\alpha$ -L-arabinofuranoside (**2a**) (2.04 g, 3.31 mmol) was dissolved in anhydrous dichloromethane and methanol, reacted with potassium carbonate (1.02 g) and purified to afford the product (**3a**) as an off-white powder.

Yield (1.12 g, 69%). mp: 167–178 °C,  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-d}_6$ )  $\delta$ : 8.94 (1H, s, C3-H), 7.81 (1H, d,  $J = 12.5$  Hz, C10-H), 4.95–4.91 (1H, m, C18-H), 4.91–4.82 (1H, m, C5-H), 3.83–3.79 (1H, m, C19-H), 3.76 (1H, m, C21-H), 3.64 (1H, m, C20-H), 3.61–3.54 (1H, m, C22-Ha), 3.42 (1H, dd,  $J = 12.5, 6.0$  Hz, C22-Hb), 3.37–3.07 (5H, m, C7-Ha, C13-H<sub>2</sub> and C14-H<sub>2</sub>), 3.03–2.81 (1H, m, C7-Hb), 2.16–1.86 (3H, m, C6-H<sub>2</sub>, C15-H<sub>2</sub> or C17-H<sub>2</sub>), 1.82–1.50 (2H, m, C15-H<sub>2</sub> or C17-H<sub>2</sub>), 1.41 (3H, d,  $J = 6.5$  Hz, C12).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-d}_6$ )  $\delta$ : 206.32 (ketone C=O), 176.49, 166.35 (carboxyl C=O), 147.56 (C3), 133.81 (aromatic C), 109.06 (C10), 106.17 (C18), 83.54 (C21), 82.58 (C19), 77.09 (C20), 61.25 (C22), 57.10 (C5), 49.40 (C13 and C14), 33.70 (C15 or C17), 30.64 (C6), 19.62 (C12), 18.63 (C7). FT-IR  $\nu$   $\text{cm}^{-1}$ : 3343.32 (m, alcohol OH), 2936.72 (w, carboxyl OH), 1716.43 (carboxylic or ester C=O), 1618.00 (aromatic C=C). MS +ESI found 493.1568 ( $\text{MH}^+$ )  $\text{C}_{24}\text{H}_{30}\text{FN}_2\text{O}_8$  required 493.1986. HPLC analysis: retention time: 5.912 min, 97.4%.

#### Nadifloxacin $\beta$ -D-galactopyranoside (**3b**)

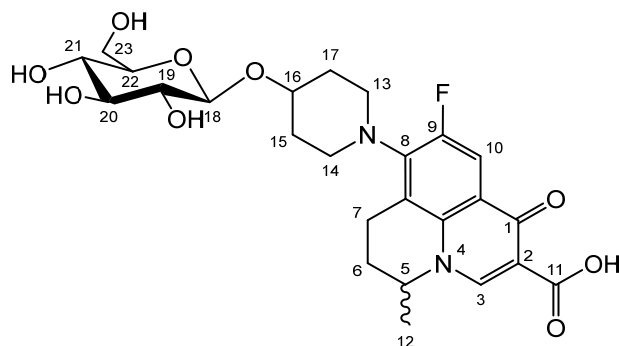




Nadifloxacin 2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-galactopyranoside (**2b**) (2 g, 2.90 mmol) was dissolved in anhydrous dichloromethane and methanol, reacted with potassium carbonate (1 g) and purified to afford the product (**3b**) as an off-white powder.

Yield (1.12 g, 74%), m.p. 149–161 °C.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 8.95 (1 H, d,  $J = 3.0$  Hz, C3-H), 7.84 (1 H, dd,  $J = 12.5, 3.0$  Hz, C10-H), 4.96–4.83 (1H, m, C5-H), 4.28–4.2 (1H, m, C18-H), 3.66–3.60 (1H, m, C20-H), 3.58–3.43 (2H, m, C23-H<sub>2</sub>), 3.44–3.20 (2H, m, C19-H, C21-H, C22-H), 3.11 (1H, d,  $J = 16.5$  Hz, C7-Ha), 3.02–2.83 (1H, m, C7-Hb), 2.20–1.97 (2H, m, C6-H<sub>2</sub>), 2.02–1.53 (4H, m, C15-H<sub>2</sub> and C17-H<sub>2</sub>), 1.41 (3H, d,  $J = 4.5$  Hz, C12-H<sub>3</sub>).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$ : 166.08 (carboxyl C=O), 147.25 (C3), 133.46 (aromatic C), 108.99 (C10), 101.56 (C18), 75.09 (C22), 73.19, 70.39 (C19 and C21), 67.95 (C20), 60.45 (C23), 57.02 (C5), 33.66 (C15 and C17), 24.80 (C6), 19.55 (C12), 18.53 (C7). FT-IR  $\nu$   $\text{cm}^{-1}$ : 3383 (w, alcohol C-OH), 2925 (w, carboxylic C-OH), 1698 (m, ketone C=O), 1628 (m, aromatic C=C), 1448 (m, alkyl C-H), 1021 (s, C-O). MS + ESI found 523.2897 (MH<sup>+</sup>) C<sub>25</sub>H<sub>32</sub>FN<sub>2</sub>O<sub>9</sub> required 523.2092. HPLC analysis: retention time: 3.042 min, purity: 97.3%.

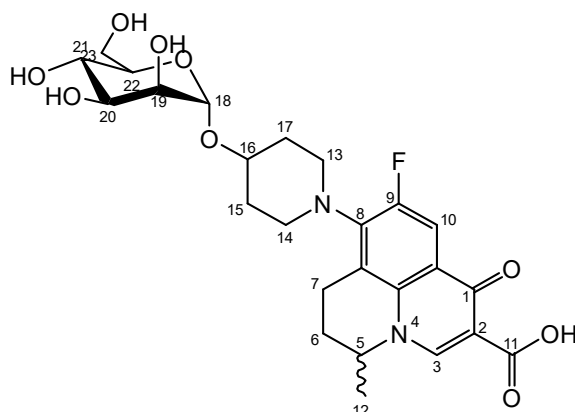
#### Nadifloxacin $\beta$ -D-glucopyranoside (**3c**)



Nadifloxacin 2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranoside (**2c**) (2.6 g, 3.77 mmol) was dissolved in anhydrous dichloromethane and methanol, reacted with potassium carbonate (1.3 g) and purified to afford the product (**3c**) as an off-white powder.

Yield (1.26 g, 64%), m.p. 162–163 °C,  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 8.94 (1H, s, C3-H), 7.83 (1H, d,  $J = 12.0$  Hz, C10-H), 4.93–4.80 (1H, m, C5-H), 4.30 (1H, d,  $J = 7.0$  Hz, C18-H), 3.88 (2H, s, C13-H<sub>2</sub> or C14-H<sub>2</sub>), 3.67 (1H, d,  $J = 11.5$  Hz, C23-Ha), 3.43 (1H, d,  $J = 9.5$  Hz, C23-Hb), 3.23 (2H, s, C13-H<sub>2</sub> or C14-H<sub>2</sub>), 3.20–3.06 (4H, m, C20-H, C21-H, C22-H and C7-Ha), 2.96 (1H, at,  $J = 7.0$  Hz, C19-H), 2.94–2.85 (1H, m, C7-Hb), 2.20–1.98 (2H, m, C6-H<sub>2</sub>), 1.85–1.48 (4H, m, C15-H<sub>2</sub> and C17-H<sub>2</sub>), 1.41 (3H, d,  $J = 5.5$  Hz, C12-H<sub>3</sub>).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$ : 166.08 (carboxyl C=O), 147.10 (C3), 133.48 (aromatic C), 133.16 (aromatic C), 106.24 (C10), 100.41 (C18), 76.8 (C20, C22), 73.52 (C19), 70.45 (C21), 61.06 (C23), 57.04 (C5), 49 (C13 or C14) 33.60 (C15 and C16), 19.56 (C12), 18.60 (C7). FT-IR  $\nu$   $\text{cm}^{-1}$ : 3386 (w, b, alcohol C-OH), 2925 (w, carboxylic C-OH), 1703 (m, ketone C=O), 1628 (m, aromatic C=C), 1448 (m, alkyl C-H), 1021 (s, C-O). MS + ESI found 523.2073 (MH<sup>+</sup>) C<sub>25</sub>H<sub>32</sub>FN<sub>2</sub>O<sub>9</sub> required 523.2092. HPLC analysis: retention time: 2.537 min, purity: 97.7%.

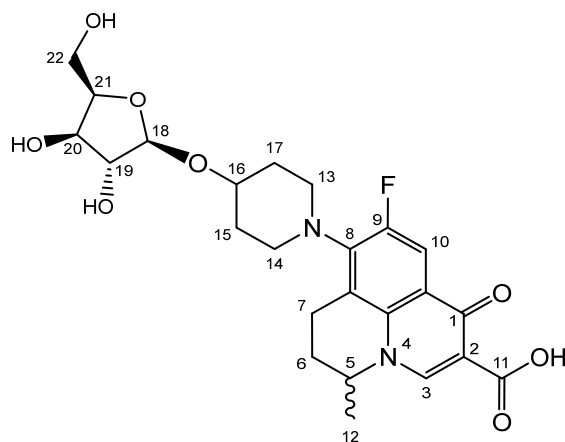
### Nadifloxacin $\alpha$ -D-mannopyranoside (3d)



Nadifloxacin 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-mannopyranoside (**2d**) (2.0 g, 2.90 mmol) was dissolved in anhydrous dichloromethane and methanol, reacted with potassium carbonate (1.0 g) and purified to afford the product (**3d**) as an off-white powder.

Yield (1.10 g, 73%). m.p. 186 °C,  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 8.94 (1H, s, C3-H), 7.84 (1H, d,  $J = 12.5$  Hz, C10-H), 4.92–4.81 (2H, m, C5-H and C18-H), 3.83 (2H, as, C13-H<sub>2</sub> or C14-H<sub>2</sub>), 3.70–3.62 (1H, m, C23-Ha), 3.62–3.58 (1H, m, C19-H), 3.55–3.30 (5H, m, C20-H, C21-H, C22-H, C23-Ha), 3.25–3.09 (1H, m, C7-Ha), 3.02–2.82 (1H, m, C7-Hb), 2.16–1.88 (4H, m, C6-H<sub>2</sub> and C15-H<sub>2</sub> or C17-H<sub>2</sub>), 1.80–1.52 (2H, m, C15-H<sub>2</sub> or C17-H<sub>2</sub>), 1.46–1.35 (3H, m, C12-H<sub>3</sub>).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$ : 165.62 (aromatic C), 147.46 (C3), 133.75 (aromatic C), 109.03 (C10), 98.23 (C18), 74.27, 70.89, 70.77, 67.05 (C20, C21 and C22), 61.37 (C23), 56.95 (C5), 33.39 (C15 and C17), 19.58 (C12), 18.52 (C7). FT-IR  $\nu$   $\text{cm}^{-1}$ : 3429.13, 3343.67 (m, alcohol OH), 2937.98 (w, carboxyl OH), 1722.74 (carboxylic or ester C=O), 1616.53 (aromatic C=C). MS +ESI found 523.2296 (MH<sup>+</sup>) C<sub>25</sub>H<sub>32</sub>FN<sub>2</sub>O<sub>9</sub> required 523.2092. HPLC analysis: retention time: 6.923 min, purity: 98.1%.

### Nadifloxacin $\beta$ -D-xylofuranoside (3e)



Nadifloxacin 2,3,5-tri-*O*-acetyl- $\beta$ -D-xylofuranoside (**2e**) (2.31 g, 3.74 mmol) was dissolved in anhydrous dichloromethane and methanol, reacted with potassium carbonate (1.16 g) and purified to afford the product (**3e**) as an off-white powder.

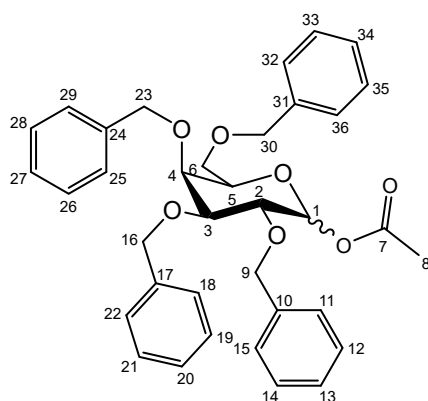
Yield (1.10 g, 66%). m.p. 145 °C,  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 9.01 (1H, s, C3-H), 7.90 (1H, d,  $J = 12.5$  Hz, C10-H), 5.00–4.91 (1H, m, C5-H), 4.34 (1H, d,  $J = 7.5$  Hz, C18-H), 3.75 (1H, m, C22-Ha), 3.54–3.26 (1H, m, C21-H), 3.22–3.10 (3H, m, C20-H, C22-Hb, C7-Ha), 3.07–2.93 (2H, m, C19-H and C7-Hb), 2.26–1.92 (4H, m, C6-H<sub>2</sub>, C15-H<sub>2</sub> or C17-H<sub>2</sub>), 1.87–1.61 (2H, m, C15-H<sub>2</sub> or C17-H<sub>2</sub>), 1.48 (3H, d,  $J = 4.0$  Hz, C12-CH<sub>3</sub>).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$ : 147.79 (C3), 133.75 (aromatic C), 109.09 (C10), 102.3 (C18), 76.65 (C20), 73.28 (C19), 69.62 (C21), 65.65 (C22), 33.77 (C15 and C17), 24.91 (C6), 19.65 (C12), 18.85 (C7). FT-IR  $\nu$   $\text{cm}^{-1}$ : 3335.48 (m, alcohol OH), 2933.13 (w, carboxyl OH), 1614.51 (aromatic C=C). MS +ESI found

493.1762 (MH<sup>+</sup>) C<sub>24</sub>H<sub>30</sub>FN<sub>2</sub>O<sub>8</sub> required 493.1986. HPLC analysis: retention time: 8.713 min, purity: 98.7%.

#### Synthesis of 1-O-acetyl-2,3,4,6-tetra-O-benzyl-D-glycosides (5a, 5b)–General method

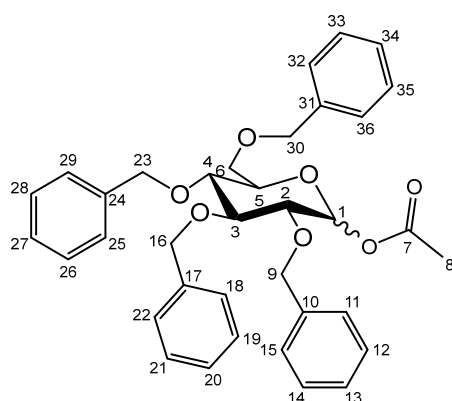
2,3,4,6-Tetra-O-benzyl-D-galactopyranose (**4a**) and 2,3,4,6-tetra-O-benzyl-D-glucopyranose (**4b**) (20 g, 37 mmol) were each dissolved in anhydrous dichloromethane (100 mL). To these solutions pyridine (6.4 mL, 74.4 mmol) and acetic anhydride (7.52 mL, 74.4 mmol) were added. The mixtures were stirred under nitrogen, monitored by TLC (hexane–ether 1:1) and stirred at room temperature for approximately 18 h. The organic solutions were extracted with 1M HCl (3 × 100 mL), sat. NaHCO<sub>3</sub> (3 × 100 mL) and brine (3 × 100 mL). The organic fractions were dried over MgSO<sub>4</sub> and concentrated in vacuo to afford an oil. The galactoside (**5a**) was precipitated from methanol to afford a white powder. The glucoside (**5b**) was used as a crude oil.

#### 1-O-Acetyl-2,3,4,6-tetra-O-benzyl-D-galactopyranoside (5a)



Yield (14.8 g, 69%), m.p. 102–104 °C, isolated as a mixture of anomers,  $\alpha$ : $\beta$  1:1.59, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.38–7.22 (m, benzyl C-H), 6.38 (d,  $J$  = 3.5 Hz,  $\alpha$ -C1-H), 5.57 (d,  $J$  = 8.0 Hz,  $\beta$ -C1-H), 4.95 (d,  $J$  = 11.5 Hz,  $\alpha$ -benzyl CH<sub>2</sub>), 4.94 (d,  $J$  = 11.5 Hz,  $\beta$ -benzyl CH<sub>2</sub>), 4.84 (d,  $J$  = 11.5 Hz,  $\beta$ -benzyl CH<sub>2</sub>), 4.82 (d,  $J$  = 11.5 Hz,  $\alpha$ -benzyl CH<sub>2</sub>), 4.74 (d,  $J$  = 11.5 Hz,  $\alpha$ -benzyl CH<sub>2</sub>), 4.75–4.69 (m, benzyl CH<sub>2</sub>), 4.62 (d,  $J$  = 11.5 Hz,  $\beta$ -benzyl CH<sub>2</sub>), 4.57 (d,  $J$  = 11.5 Hz,  $\alpha$ -benzyl CH<sub>2</sub>), 4.46 (d,  $J$  = 11.5 Hz,  $\alpha$ -benzyl CH<sub>2</sub>), 4.43 (d,  $J$  = 11.5 Hz,  $\beta$ -benzyl CH<sub>2</sub>), 4.39 (d,  $J$  = 11.5 Hz,  $\alpha$ -benzyl CH<sub>2</sub>), 4.38 (d,  $J$  = 11.5 Hz,  $\beta$ -benzyl CH<sub>2</sub>), 4.16 (dd,  $J$  = 10.0, 3.5 Hz,  $\alpha$ -C2-H), 4.06–3.99 (m, C5-H), 3.94 (m,  $\beta$ -C2-H), 3.89 (dd,  $J$  = 10.0, 2.5 Hz,  $\alpha$ -C3-H), 3.72–3.66 (m,  $\beta$ -C4-H), 2.11 (s,  $\alpha$ -C8-H<sub>3</sub>), 2.03 (s,  $\beta$ -C8-H<sub>3</sub>). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 169.63, 169.41 (carboxyl C=O), 138.64, 138.52, 138.42, 138.22, 138.06, 137.80, 137.77 (benzyl *ipso* C), 128.63–127.36 (benzyl CH), 94.30 ( $\beta$ -C1), 90.81 ( $\alpha$ -C1), 82.42 ( $\alpha$ -C5), 78.61 ( $\alpha$ -C3), 78.19 ( $\beta$ -C2), 75.42 ( $\alpha$ -C2), 75.35, 74.95, 74.72 (benzyl CH<sub>2</sub>), 74.08 ( $\beta$ -C4), 73.61, 73.54, 73.40, 73.40 (benzyl CH<sub>2</sub>), 73.04 ( $\beta$ -C5), 72.89 (benzyl CH<sub>2</sub>), 68.41 ( $\alpha$ -C6), 67.94 ( $\beta$ -C6), 21.24 ( $\alpha$ -C8), 21.06 ( $\beta$ -C8). FT-IR  $\nu$  cm<sup>-1</sup>: 3059.70, 3026.72 (w, aromatic C-H), 1747.65 (m, ester C=O), 1081.32, 1045.51, 1024.29 (s, ether C-O). MS + ESI found 621.3333 (M + K<sup>+</sup>) C<sub>36</sub>H<sub>38</sub>KO<sub>7</sub> required 621.2249.

#### 1-O-Acetyl-2,3,4,6-tetra-O-benzyl-D-glucopyranoside (5b)

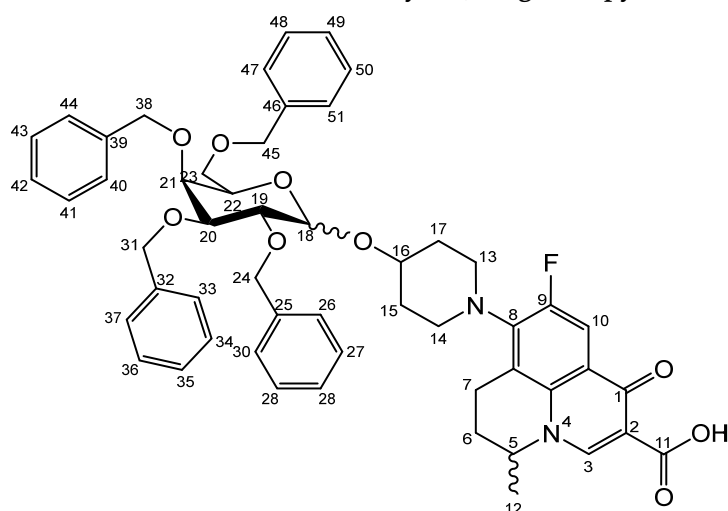


Yield (18.0 g, 83%). Isolated as a mixture of anomers,  $\alpha:\beta$  1:1.23,  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.38–7.22 (m, benzyl C-H), 7.16–7.10 (m, benzyl C-H), 6.35 (d,  $J = 3.5$  Hz,  $\alpha$ -C1-H), 5.60 (d,  $J = 8.0$  Hz,  $\beta$ -C1-H), 4.99–4.44 (m, benzyl  $\text{CH}_2$ ), 3.99–3.90 (m, glucose H), 3.89–3.83 (m, glucose H), 3.79–3.53 (m,  $\alpha$ -,  $\beta$ -C2-H and  $\alpha$ -,  $\beta$ -C6- $\text{H}_2$ ), 2.13 (s,  $\alpha$ -C8- $\text{H}_3$ ), 2.05 (s,  $\beta$ -C8- $\text{H}_3$ ).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-d}_6$ )  $\delta$ : 169.47, 169.31 (C7), 138.64, 138.11, 138.02, 137.89, 137.82, 137.60 (aromatic C), 128.58–127.59 (aromatic C-H), 94.05 ( $\alpha$ -C1), 84.82, 81.68, 81.07, 78.88, 77.22, 76.95 (glucose C), 75.72 (benzyl  $\text{CH}_2$ ), 75.40 (glucose C), 75.30, 75.04, 73.55, 73.22 (benzyl  $\text{CH}_2$ ), 72.83 (glucose C), 68.09 (C6), 21.10 (C8). FT-IR  $\nu$   $\text{cm}^{-1}$ : 3088.08, 3062.95, 3030.18 (w, aromatic C-H), 1755.44 (m, ester C=O), 1072.79, 1027.24 (s, ether C-O). MS-ESI found 621.5834 ( $\text{M} + \text{K}^+$ ) KC36H38O7 required 621.2249.

#### Synthesis of benzyl protected nadifloxacin $\alpha$ -galactoside (6a) and $\alpha$ -glucoside (6b)–General method

Nadifloxacin (**1**) (5 g, 13.9  $\mu\text{moles}$ ) was suspended in anhydrous acetone (250 mL), and to this TMSOTf was added (2.51 mL, 13.9  $\mu\text{moles}$ ), and the solution was stirred. To this the 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-glycoside (**5a** or **5b**) (16.1 g, 13.9  $\mu\text{moles}$ ) was added followed by TMSOTf (22.59 mL, 124.9  $\mu\text{moles}$ ). Reactions were stirred at room temperature under an atmosphere of nitrogen for 24 h. The reaction mixtures were then diluted with anhydrous dichloromethane (250 mL), washed with sat.  $\text{NaHCO}_3$  ( $2 \times 100$  mL) and brine ( $2 \times 100$  mL), dried over  $\text{MgSO}_4$  and concentrated in vacuo. The products (**6a**) or (**6b**) were purified on silica columns and eluted with toluene (A) and acetonitrile (B) at 14% B to 36% B over 5 column volumes and maintained at 36% for 10-column volumes.

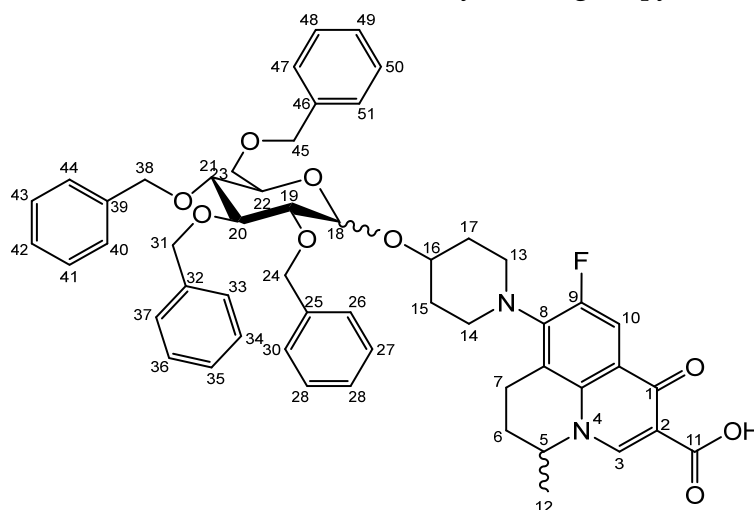
#### Nadifloxacin 2,3,4,6-tetra-*O*-benzyl- $\alpha,\beta$ -D-galactopyranoside (6a)



Yield (6.83 g, 56%). m.p. 61–65  $^\circ\text{C}$ , isolated as a mixture of anomers,  $\alpha:\beta$  3:5,  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.63 (1H, s, C3-H), 7.93 (1H, d,  $J = 12.0$  Hz, C10-H), 7.36–7.15 (m, benzyl C-H), 4.98–4.94 (m,  $\alpha$ -C18-H), 4.94–4.84 (m, benzyl  $\text{CH}_2$ ), 4.83–4.46 (m, benzyl  $\text{CH}_2$ , C5-H), 4.44 (d,  $J = 8.5$  Hz,  $\beta$ -C18-H), 4.41–4.30 (m, benzyl  $\text{CH}_2$ ), 4.03–3.95 (m,  $\alpha$ -C19-H

and C22-H), 3.95–3.88 (C21-H), 3.82 (d,  $J = 9.5$  Hz, C22-H), 3.77 (d,  $J = 9.0$  Hz,  $\beta$ -C19-H), 3.52–3.44 (m, C20-H and C23-H<sub>2</sub>), 3.21 (d,  $J = 17.0$  Hz, C7-Ha), 3.03–2.90 (m, C16-H), 2.85–2.67 (m, C7-Hb), 2.15–2.03 (m, C6-H<sub>2</sub>), 2.03–1.59 (m, C15-H<sub>2</sub> and C17-H<sub>2</sub>), 1.48–1.39 (m, C12-H<sub>3</sub>). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 167.16 (carboxyl C=O), 146.47 (C3), 128.62–127.27 (aromatic C), 125.29 (aromatic C), 110.72 (C10), 101.92 ( $\beta$ -C18), 5.83 ( $\alpha$ -C18), 82.12 (C20), 79.77 ( $\beta$ -C19), 79.27 (C21), 76.06 ( $\alpha$ -C19), 75.32 (benzyl CH<sub>2</sub>), 75.03 (C21), 74.77 (benzyl CH<sub>2</sub>), 74.53 (benzyl CH<sub>2</sub>), 73.94 (C20), 73.65 (C22) 73.13 (benzyl CH<sub>2</sub>), 69.78 (C22), 69.31, 69.01 (C23), 32.64 (C15, C17), 26.04 (C6), 20.25 (C12), 18.93 (C7). FT-IR  $\nu$  cm<sup>-1</sup>: 3061.62, 3029.55 (w, aromatic C-H), 2920.54, 2861.87 (w, carboxylic C-OH), 1723.88 (m, carbonyl C=O), 1619 (m, C=C), 1490 (s, arene C-C), MS +ESI found 883.3593 (MH<sup>+</sup>) C<sub>53</sub>H<sub>56</sub>FN<sub>2</sub>O<sub>9</sub> expected 883.3970.

#### Nadifloxacin 2,3,4,6-tetra-*O*-benzyl- $\alpha,\beta$ -D-glucopyranoside (6b)

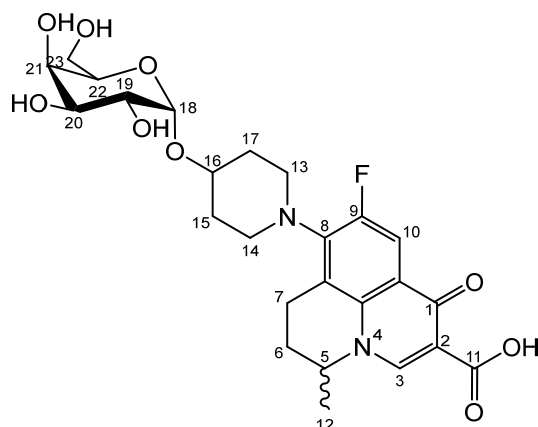


Yield (1.75 g, 14%), <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.69 (s, C3-H), 8.00 (d,  $J = 12.0$  Hz, C10-H), 7.41–7.21 (m, benzyl H), 5.06–4.96 (m,  $\alpha$ -C18-H, benzyl CH<sub>2</sub>), 4.95–4.72 (m, benzyl CH<sub>2</sub>), 4.71–4.44 (benzyl CH<sub>2</sub>,  $\beta$ -C18-H, C5-H), 4.03 (at,  $J = 9.5$  Hz,  $\alpha$ -C20-H), 3.94–3.87 (m, glucose H), 3.78–3.71 (m, C23-Ha), 3.71–3.44 (m, C23-Hb,  $\alpha$ -C19-H,  $\beta$ -C19-H, glucose H), 3.38–3.16 (m, C7-Ha), 3.15–3.00 (m, C13-H<sub>2</sub> or C14-H<sub>2</sub>), 2.93–2.75 (m, C7-Hb), 2.11–1.64 (m, C17-H<sub>2</sub>, C15-H<sub>2</sub>), 1.53 (d,  $J = 6.5$  Hz, C12-H<sub>3</sub>), 1.52 (d,  $J = 6.5$  Hz, C12-H<sub>3</sub>). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 177.14, 167.35 (carbonyl C=O), 110.77, 110.49 (C10), 101.88 ( $\beta$ -C18), 95.13 ( $\alpha$ -C18), 84.52, (glucose C), 82.29 ( $\beta$ -C19), 82.04 ( $\alpha$ -C20), 79.98, 77.88 (glucose C), 75.69, 75.25, 75.04, 74.95 (benzyl CH<sub>2</sub>), 74.82 ( $\beta$ -glucose C), 73.50, 73.42, 73.33, 73.25 (benzyl CH<sub>2</sub>), 70.51 (glucose C), 68.62 (C23), 57.95 (C5), 33.12 (C17, C15), 30.97 (C6), 20.23 (C12), 18.83 (C7). FT-IR  $\nu$  cm<sup>-1</sup>: 3062.43, 3028.98 (w, aromatic C-H), 2900.68, 2865.08 (w, carboxylic C-OH), 1618.42 (m, C=C), 1452 (s, arene C-C), 1067.21, 1027.67 (s, C-O). MS + ESI found 883.3850 (MH<sup>+</sup>) C<sub>53</sub>H<sub>56</sub>FN<sub>2</sub>O<sub>9</sub> expected 883.3970.

#### Deprotection of per-*O*-benzylated nadifloxacin glycosides and resolution of anomers by enzyme hydrolysis (7a, 7b)–General method

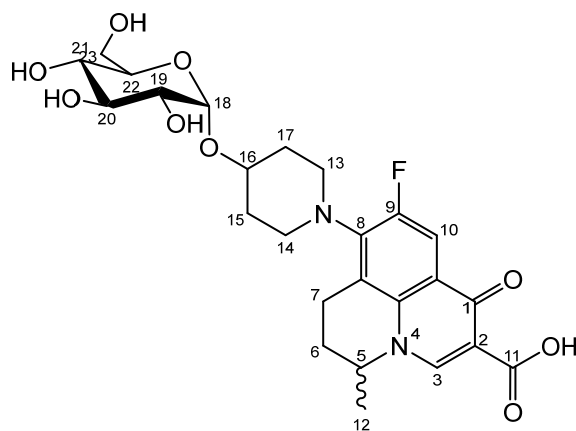
De-*O*-benzylation of glycosides (6a) and (6b) was achieved using Pd/C (10 wt.%) in methanol under an atmosphere of hydrogen. After 24 h, the mixtures were filtered over Celite and concentrated in vacuo to afford anomeric mixture of nadifloxacin glycosides (7a  $\alpha,\beta$ ) and (7b  $\alpha,\beta$ ). These were then stirred in a suitable buffer,  $\beta$ -glycosidase (100 U) was added for 7a  $\alpha,\beta$ :  $\beta$ -galactosidase from *E. coli*, for 7b  $\alpha,\beta$ :  $\beta$ -glucosidase from almonds, and the suspensions were incubated at 37 °C and monitored by HPLC analysis for the decrease in the  $\beta$ -glycoside. Once the  $\beta$ -anomers had all been hydrolysed, the reaction mixtures were filtered, adjusted to pH 3.0 using formic acid and purified on C18 columns and eluted with 0.1% (*v/v*) formic acid in water (A) and acetonitrile (B) (70:30).

#### Nadifloxacin $\alpha$ -D-galactopyranoside (7a)



Yield (1.25 g, 33%), m.p. 143–150 °C,  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 8.94 (1H, s, C3-H), 7.83 (1H, d,  $J$  = 12.0 Hz, C10-H), 4.99–4.76 (2H, m, C18-H and C5-H), 3.76–3.67 (3H, galactose C-H), 3.63–3.55 (2H, m, C19-H and galactose C-H), 3.54–3.40 (2H, m, C23- $\text{H}_2$ ), 3.47–3.17 (4H, m, C13- $\text{H}_2$  and C14- $\text{H}_2$ ), 3.03–2.83 (2H, m, C7- $\text{H}_2$ ), 2.21–1.96 (2H, m, C6- $\text{H}_2$ ), 2.00–1.54 (4H, m, C15- $\text{H}_2$  and C17- $\text{H}_2$ ), 1.41 (3H, d,  $J$  = 5.0 Hz, C12- $\text{H}_3$ ).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$ : 166.08 (Carboxyl C=O), 147.25 (C3), 133.50 (Aromatic C), 108.87 (C10), 106.28 (Aromatic C), 97.64 (C18), 71.45, 69.55, 68.87, 68.24 (galactose C), 60.57 (C23), 57.06 (C5), 33.08 (C15 and C17), 24.76 (C7), 19.57 (C12), 18.51 (C6). FT-IR  $\nu$   $\text{cm}^{-1}$ : 3373.83 (m, alcohol OH), 2934.33 (w, carboxyl OH), 1708.29 (carboxylic or ester C=O), 1620.84 (aromatic C=C). MS + ESI found 523.2216 ( $\text{MH}^+$ )  $\text{C}_{25}\text{H}_{32}\text{FN}_2\text{O}_9$  required 523.2092. HPLC analysis: retention time: 4.127 min, purity: 98.2%.

#### Nadifloxacin $\alpha$ -D-glucopyranoside (7b)



Yield (0.20 g, 27%), m.p. 162 °C,  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 9.01 (1H, s, C3-H), 7.90 (1H, d,  $J$  = 12.0 Hz, C10-H), 5.03–4.83 (2H, m, C5-H and C18-H), 3.69 (1H, d,  $J$  = 11.0 Hz, C23-Ha), 3.61–3.46 (3H, m, C20-H, C22-H, C23-Hb), 3.30–3.20 (1H, m, C19-H), 3.25–3.12 (1H, m, C6-Ha), 3.20–3.08 (1H, m, C21-H), 3.07–2.88 (1H, m, C6-Hb), 2.25–1.95 (2H, m, C7- $\text{H}_2$ ), 2.07–1.60 (4H, m, C15- $\text{H}_2$  and C17- $\text{H}_2$ ), 1.48 (3H, d,  $J$  = 7.0 Hz).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$ : 176.47 (carboxyl C=O), 166.08 (aromatic C), 147.40 (C3), 133.61 (aromatic C), 108.99 (C10), 106.44 (aromatic C), 97.28 (C18), 73.16 (C20), 73.05 (C22), 71.81 (C19), 70.37 (C21), 61.03 (C23), 57.07 (C5), 24.84 (C7), 19.57 (C12), 18.53 (C15 and C17). FT-IR  $\nu$   $\text{cm}^{-1}$ : 3359.86 (m, alcohol OH), 2928.52 (w, carboxyl OH), 1709.04 (carboxylic or ester C=O), 1621.75 (aromatic C=C). MS + ESI found 523.1920 ( $\text{MH}^+$ )  $\text{C}_{25}\text{H}_{32}\text{FN}_2\text{O}_9$  required 523.2092. HPLC analysis: retention time: 5.028 min, purity 98.4%.

#### Determination of minimum inhibitory concentration

The glycosides (3a-e and 7a,b) and underivatized nadifloxacin (1) were individually dissolved in dimethyl sulfoxide, and amounts of the solutions were added to Tryptone Soya Agar (TSA) that had been autoclaved and then cooled to 50 °C to produce doubling

concentrations of nadifloxacin (**1**) from 0.125–8 µg/mL and 1–128 µg/mL of the glycosides (**3a-f** and **7a,b**). The agar was swirled to mix, and then plates were poured and dried. Specifically, a stock of nadifloxacin (**1**) was prepared in DMSO (1.6 mg/mL). Concentrations of 800, 400, 200, 100, 50 and 25 µg/mL were prepared by serial dilutions starting with addition of 0.5 mL of the 1.6 mg/mL solution into 0.5 mL of DMSO and so forth. An aliquot (100 µL) from each concentration was added individually to molten agar (20 mL) to give final agar concentrations of 8, 4, 2, 1, 0.5, 0.25 and 0.125 µg/mL. A stock of each nadifloxacin glycoside was prepared in DMSO (25.6 mg/mL). Concentrations of 12.8, 6.4, 3.2, 1.6, 0.8, 0.4 and 0.2 mg/mL were prepared by serial dilutions starting with addition of 0.5 mL of the 25.6 mg/mL solution into 0.5 mL of DMSO. A nadifloxacin glycoside concentration of 20 µg/mL was prepared by diluting 100 µL of concentration 0.2 mg/mL into 900 µL of DMSO. An aliquot (100 µL) from each concentration was added individually to molten agar (20 mL) to give final agar concentrations of 128, 64, 32, 16, 8, 4, 2, 1 and 0.1 µg/mL. A plate containing no nadifloxacin glycoside was used as control. Plates were cooled and dried before use. A plate containing no nadifloxacin was used as control. Plates were cooled and dried before use.

Cultures of bacteria were grown overnight in Nutrient Broth 2 (NB2) at 37 °C from beads stored at –80 °C. Cultures were then diluted decimally in Maximum Recovery Diluent (MRD) to approximately 10<sup>6</sup> CFU/mL, and 300 µL of each organism added to the wells of a multipoint inoculator (“Oxoid Cathra Replicator”, ThermoFisher Scientific). Pins of the inoculator then dispensed amounts of bacterial suspension onto the surface of the agar plates. Plates were incubated at 37 °C and inspected after 18 h for growth to obtain cultures in the log phase, wherein cells were actively dividing. The lowest amount of antibacterial agent that totally inhibited growth was recorded as the MIC for that organism.

#### 4. Conclusions

The poor aqueous solubility of nadifloxacin (**1**) has limited its clinical use to topical applications, and this is unfortunate since it exhibits broad-spectrum antibacterial activity at very low concentrations. The glycosides prepared in this study were water-soluble, meaning that aqueous-based preparations are now feasible. Importantly, all of the *Staphylococcus aureus* strains, including four MRSA strains, were found to be sensitive to, or intermediately sensitive to, the glycosides. Hence, the glycosides could be suitable as lead compounds for the development of orally administered antibacterial agents for the treatment of MRSA infections. The stability of the glycosidic bond should also allow them to arrive at the site of infection intact. The most potent glycoside, nadifloxacin- $\alpha$ -L-arabinofuranoside (**3a**), had MICs of 1–8 µg/mL for the eight *S. aureus* strains, making it a prime candidate for further studies. Since the glycosides exhibited different MICs for different organisms, they could also be developed as tools for the selective isolation of target organisms in the presence of background flora.

**Author Contributions:** Conceptualization, all authors; Methodology, all authors; Validation, all authors.; Formal analysis, M.H.; Investigation, M.H.; Writing—original draft preparation, M.H., R.A.B., H.M.I.O.; Writing—review and editing, all authors; Supervision, J.A.B., R.A.B., H.M.I.O., P.J.S.; Project administration, J.A.B., R.A.B., H.M.I.O., P.J.S.; Funding acquisition, R.A.B., M.H., P.J.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by ThermoFisher Scientific.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** The authors are grateful to ThermoFisher Scientific for their financial support. We also thank the University of Reading for the provision of the Chemical Analysis Facility.

**Conflicts of Interest:** The authors declare no conflict of interest.

**Sample Availability:** Not available.

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