

# Regioselective Glycosylation of Novobiocin Alters Activity

Guoxuan Sun,<sup>a</sup> Pedro Ernesto de Resende,<sup>a</sup> Paul Stapleton,<sup>a</sup> Martyna Kuta,<sup>a</sup> Xiangtao Wang,<sup>b</sup> Shozeb Haider,<sup>a</sup> and Min Yang<sup>a\*</sup>

a. The School of Pharmacy, University College London, 29/39 Brunswick Square, London, WC1N 1AX, UK

b. The Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, 100193, China

Corresponding author: [min.yang@ucl.ac.uk](mailto:min.yang@ucl.ac.uk)

**Abstract:** Glycosylation is a promising approach to overcome antimicrobial drug resistance. In this study, we investigated Koenigs-Knorr and phase transfer glycosylation on novobiocin. While the former only gave a 4'-OH product, the later produced mainly a kinetic controlled 5-OH product, but still achieved the 4'-OH modification and novobiocin-glycosylated products (with stronger base), as well as a diglycosylated compound. Investigation on the antibacterial activity indicate that the presence of galactose moiety helps to improve activity possibly via enhanced cellular uptake.

## 1. Introduction

Antimicrobial agents are the last line of defence against microorganisms. Overuse and/or misuse of antibiotics has been linked to resistant organisms such as multidrug resistant (MDR) Gram-negative bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Such organisms are responsible for 25,000 deaths each year and more than €1.5 billion in healthcare-associated expenses and productivity losses in Europe alone.[1] Consequently, efforts have now hastened to find new strategies to address the increasing resistance problem; one promising approach is to modify a compound by glycosylation.[2] Many antibiotics are glycosylated and contain a variety of different sugars.[3-7] Glycosylation modifies antimicrobial agent pharmacokinetic parameters such as solubility, membrane transport, pharmacodynamics, mechanism and potency.[8] The sugar residues can increase or decrease potency[9, 10] either by being directly involved in the binding process [11, 12] or by being essential for antimicrobial activity [13].

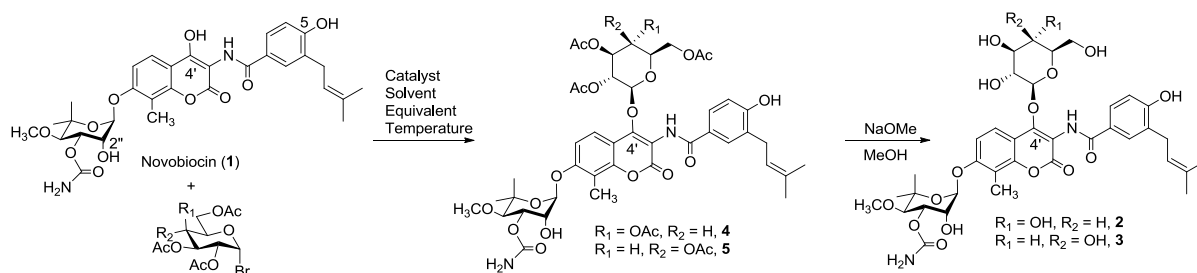
Novobiocin is a member of the aminocoumarin family and acts as a bacterial DNA synthesis inhibitor by binding to the B subunit of the DNA gyrase.[14] It also possess anticancer activity by binding to the HSP90 chaperone.[15, 16] The clinical use of this class of antibiotics has been restricted due to their low water solubility, low activity against Gram-negative bacteria [17] and toxicity *in vivo*.[18] Modifications of aminocoumarins have been made to achieve better activity in the past decade.[19-22] Previously we discovered that novobiocin can be glycosylated by glycosyltransferases. This simple

glycosylation separates the anticancer activity from antibacterial activity up to  $2.67 \times 10^4$  fold.[23] However, the exact mechanism is not fully clear.

## 2. Result and discussion

### 2.1 Phase transfer glycosylation

Previously, the glycosylated novobiocins were obtained either via enzymatic catalyzed synthesis, which was difficult to scale up or involves the use of mercury cyanide which is extremely toxic. In order to find an alternative synthetic route, we tested a few conditions, such as Koenigs-Knorr (Fig. 1, Table 1) and phase transfer glycosylation ( Fig. 2, Table 2) with different solvents, catalysts and temperature. In the Koenigs-Knorr reactions, only DMF, a polar aprotic solvent, gave reasonable yield (31%) with the tetra-acetylated glucosyl bromide donor and novobiocin due to its strong solvent effect and capacity to fully solubilise novobiocin. Adding catalyst or increase temperature led to a decrease the yield (Table 1) probably because the reaction was slow and a fast forming oxinium intermediate was not in favour of the desired product. However, it should be noted that under these conditions, only glycosylation on the 4'-OH was formed (Fig. 1, Fig. S1).



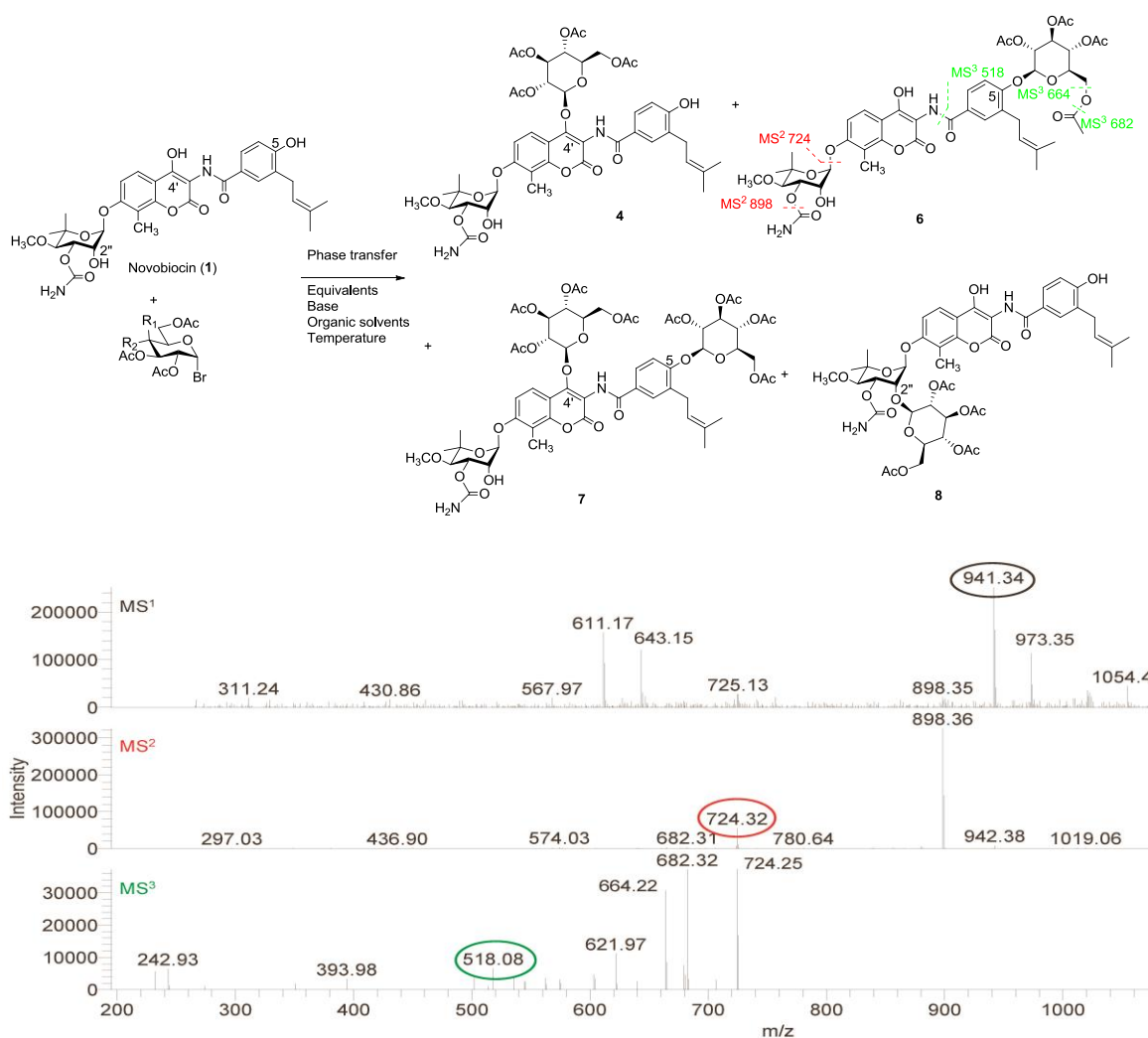
**Figure 1.** Glycosylation with Koenigs-Knorr condition.

**Table 1.** Glycosylation of novobiocin with various conditions.

No	Solvent	Catalyst	Eqv.	Temp/°C	RT /min	Mass (ESI <sup>+</sup> )	Yield
1	THF	Hg(CN) <sub>2</sub>	1.2	rt	3.78	943	57%
2	THF	AgBF <sub>4</sub>	1.1	rt	-	-	-
3	THF	AgOTf	1.1	rt	3.82	-	Trace
4	THF	CuOTf	1.1	rt	-	-	-
5	DMF	no	1.1	rt	3.82	943	31%
6	DMF	no	1.1	45	3.81	943	14%
7	DMF	AgCO <sub>3</sub>	1.1	rt	3.82	943	17%
8	DMF	BiONO <sub>3</sub>	1.2	rt	3.81	943	11%
9	DMF	AgOTf	1.1	rt	3.81	943	10%
10	DMF	K <sub>2</sub> CO <sub>3</sub>	1.1	rt	3.80	943	9%
11	DMF	LiBr	1.1	rt	-	-	-
12	PC	no	1.1	rt	3.79	943	15%

Eqv: Equivalent. rt: room temperature. RT: retention time. Yield: estimated from LC UV spectra. PC: Propylene carbonate.

Phase transfer reaction conditions (Fig. 2, Table 2) suggested that chloroform was a better solvent than DCM and that a higher equivalent of benzyl tributyl ammonium bromide (BTAB) and stronger ( $\text{CO}_3^{2-}$  vs  $\text{HCO}_3^-$ ) base favoured a better yield. However, it should be stressed that it produced a different glycosylation product with different retention time (4.6 min instead of 3.8 min, Table 2, Fig. S2). Ion trap analysis; while all major  $\text{MS}^3$  peaks could be confidently assigned, the ion of 518.08 proved a 5-OH glycosylation (Fig. 2). The structure was further validated by NMR (Table S1) - a coupling between  $\text{H1}'''$  and C5 indicated the linkage. At elevated temperature (Table 2, No 19) the 4'-OH (3.8 min) and 5-OH glycosylated products could be observed as well as a diglycosylated product (Fig. S3). As the 5-OH is more accessible but with higher pKa values [23] compared to 4'-OH, this probably means that the increase in temperature changed the reaction from kinetic to thermodynamic control. The much stronger base ( $\text{OH}^-$ ) caused decarbamation of novobiocin and its glycosylated product, but produced another glycosylated product (4.98 min) which may possibly occur on the 2''-OH in noviose (Table. 2, No 20, Fig. S4).



**Figure 2.** Glycosylation with phase transfer condition and MS/MS analysis of products. Top: reaction scheme; bottom: MS/MS analysis of compound 6 to elucidate the structure. MS<sup>1</sup>: full MS spectrum. 941.34, [M-H]<sup>+</sup>. MS<sup>2</sup>: MS/MS analysis of 941. Peaks of 898.36 and 724.32 are indicated in the reaction scheme (red cleavage). MS<sup>3</sup>: MS<sup>3</sup> analysis of 724.32 in MS<sup>2</sup>. The peak of 518.08 indicated that glucose moiety was added onto the phenol-OH (5-OH). Peaks of 682.32 and 664.22 are indicated in the scheme (Top, green cleavage). Peak of 621.97 was formed from peak 664.22 by removing one of the acetyl groups

**Table 2. Phase transfer condition for the synthesis of glucosyl-novobiocin.**

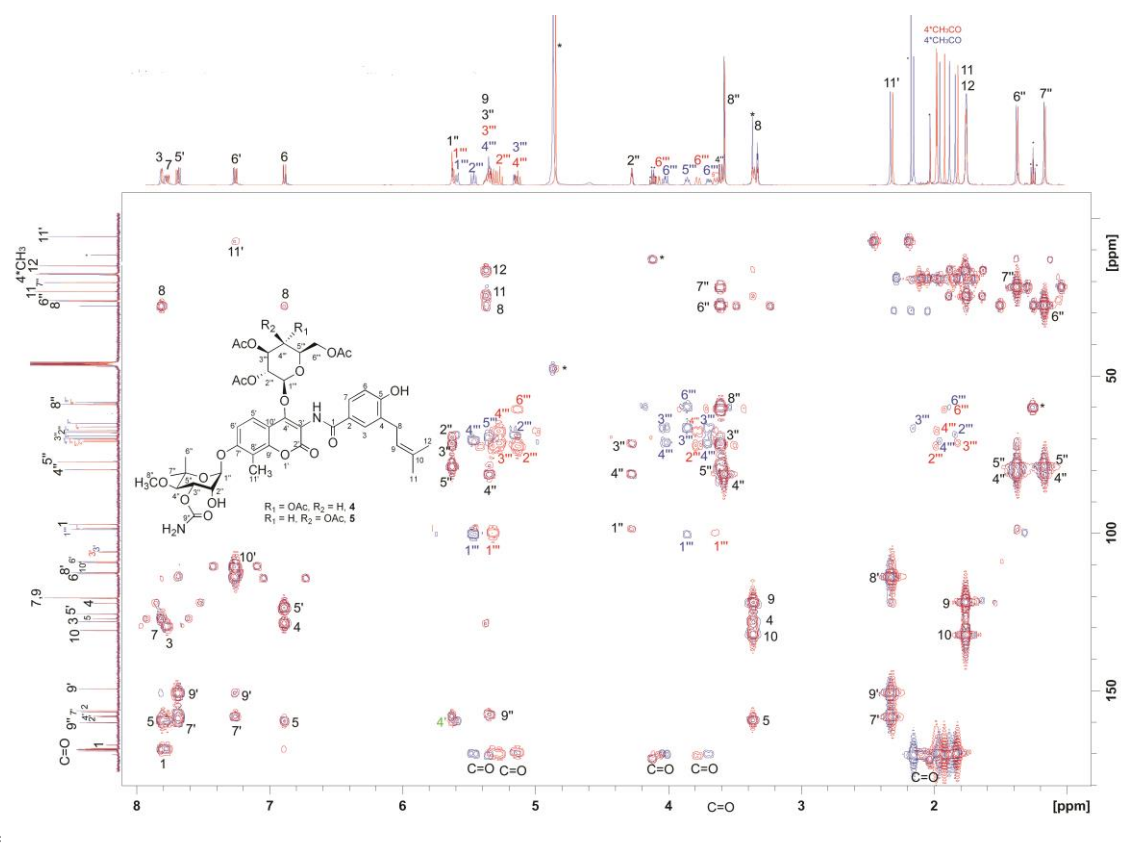
Entry	Promoter	Eqv.	Water/Organic	Donor /Eqv.	Temp/°C	RT/min	Mass (ESI <sup>+</sup> )	Yield
13	BTAB	0.1	Water/DCM	1.1	rt	-		
14	BTAB	0.1	0.1M NaHCO <sub>3</sub> /DCM	1.1	rt	-		
15	BTAB	0.1	0.15M K <sub>2</sub> CO <sub>3</sub> /DCM	1.1	rt	4.59	943	12%
16	BTAB	1	0.15M K <sub>2</sub> CO <sub>3</sub> /DCM	1.1	rt	4.60	943	16%
17	BTAB	1	0.15M K <sub>2</sub> CO <sub>3</sub> /DCM	4	rt	4.59	943	30%
18	BTAB	1	0.15M K <sub>2</sub> CO <sub>3</sub> /CHCl <sub>3</sub>	4	rt	4.60	943	45%
19	BTAB	1	0.15M K <sub>2</sub> CO <sub>3</sub> /CHCl <sub>3</sub>	4	45	3.76	943	28%
						4.10	1273	29%
						4.55	943	9%
20	BTAB	1	0.08 M KOH/CHCl <sub>3</sub>	4	rt	4.42	570	25%
						4.68	900	25%
						4.98	943	18%

Note: BTAB: Benzyltributylammonium Bromide. rt, room temperature. RT: retention time. 4-glycosylated product marked in red. Phenol-glycosylated product marked in blue. There is also an unidentified product marked in green which could be another glycoside of novobiocin due to its identical molecular weight. Yield: estimated from LC UV spectra.

In No 19, product RT=4.10 min is characterized as double-glycosylated product of novobiocin.

In No 20, product RT=4.42 min is characterized as novobiocin lost its carbamate group located on novose sugar. As a result, product RT=4.68 min is the glycoside of product RT=4.42 min.

Figure 3 shows the HMBC-NMR of compounds **4** & **5**, obtained in the presence of DMF alone. Both H1''' from a sugar coupled with C4' in the coumarin ring clearly proved the glycosylation on the 4'-OH position. While most other peaks retain the same chemical shifts both in HNMR and CNMR, the sugar moiety (red for glucose and blue for galactose) showed quite different shift and coupling constants, especially H4'''. Most three bonds long range couplings could be observed such as within the 2 ppm and 65 ppm regions, and a proton from an acetyl group coupled to 2''' to 6''' respectively, but not 5'''. Compounds **2** & **3** were obtained by deprotection of **4** & **5** respectively according to procedures reported previously. [23]



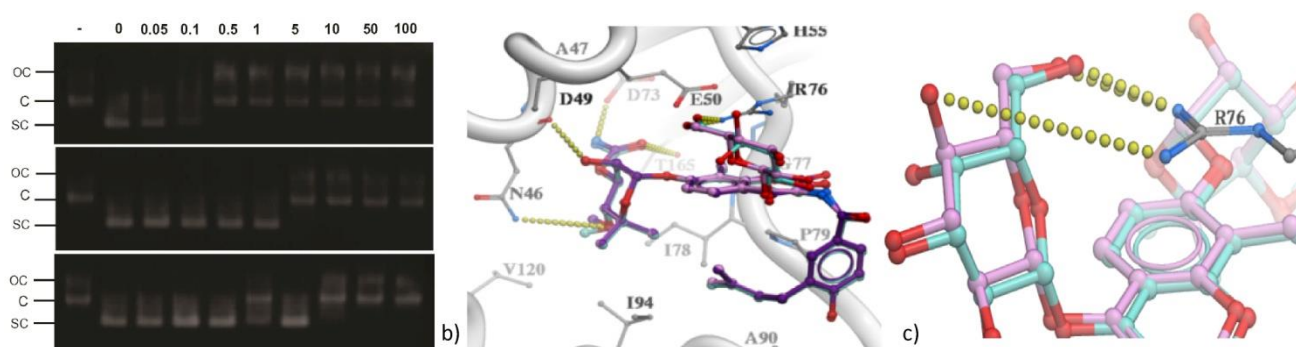
**Figure 3. Combined HMBC NMR analysis of tetra-O-acetyl-β-glucosyl-novobiocin (Red) and galactosylnovobiocin (Blue).** Both spectra indicated the binding between H1''' to 4' (Green). Long range couplings can be observed on acetyl CH<sub>3</sub> to 2''' to 6''' but not 5'''. Unlabelled peaks are <sup>1</sup>H coupled <sup>13</sup>C NMR peak. \*: solvent residues.

## 2.2 Biological activity test

Novobiocin inhibits DNA supercoiling catalyzed by DNA gyrase. DNA gyrase activity assays were carried out on **1**, **2** and **3** (Fig. 4). **1** showed inhibition at 0.5 μM, which is consistent with the value reported in the literature (0.9 μM[24]). **2** and **3** inhibited DNA gyrase at 5 and 10 μM, respectively. This indicates that adding a sugar residue on novobiocin reduced the DNA gyrase-binding capacity of the compound.

The crystal structure of the DNA gyrase (PDB id:1AJ6) was used as a receptor in docking studies using ICM-Pro Molsoft software.[25] Compound **1** was re-docked to the same position within the crystal structure, the binding energy of **1** (-25.84 kcal/mol), **2** (-21.32 kcal/mol) and **3** (-19.35 kcal/mol) are in agreement with the gyrase assay result. Compounds **2** and **3** are in the same position as compound **1**, with noviose moieties in the pocket. In all three cases, we observed hydrogen bond between ligands (noviose moieties) and residues N46, T165 and D73, which contribute to the binding pocket. The added sugar in **2** and **3** creates new hydrogen bonds with residue R76 (Fig. 4).

The *in vitro* antibacterial activities of **1-5** were evaluated against several clinically relevant bacteria (Table S2). Neither novobiocin or modified novobiocin derivatives showed strong antibacterial activity against various strains. Galactosyl-novobiocin (**3**) showed about 2-fold increased potency probably due to the enhanced uptake via permease exploiting the galactose residues, which was reported previously.[26]



**Figure 4. Antibiotics effect of compounds 1-3.** a) Effect on the capacity of DNA gyrase to supercoiled relaxed pBR322 DNA.-, relaxed pBR322 without DNA gyrase; OC, open circular plasmid DNA; C, coiled plasmid DNA; SC, supercoiled, unit:  $\mu\text{M}$ . Top: Novobiocin (**1**); Middle: glucosyl\_novobiocin (**2**) and Bottom; galactosyl\_novobiocin (**3**).[23] b) DNA gyrase protein structure (PDB ID: 1AJ6) with re-docked **1** (violet), and docked compound **2** (pink) and **3** (blue). Both **2** & **3** interact with the pocket in the same way like **1** by creating four hydrogen bonds between the noviose moiety and residues N46, T165 and D73. The glucose/galactose moieties create new hydrogen bonds with residue R76. Yellow lines: hydrogen bonds. c) A zoomed-out image of glucose/galactose binding positions from b).

### 3. Experimental

#### 3.1 General method of Koenigs-Knorr glycosylation (Table1, 1-4)

A mixture of novobiocin sodium (2.0g, 3.159mmol), 1-Bromine-2,3,4,6-tetra-O-acetyl- $\alpha$ -D-glucose/galactose (1.32g, 3.218 mmol), activated 4Å molecular sieves (approx. 6 g) and anhydrous THF (100mL) were added into a 250ml flask. Catalyst (1.0eqv) was added into the system and then the reaction mixture was stirred for 7 days under nitrogen at room temperature covered by foil and monitored by LC/MS (Shimazu 2020, Column: XTerra, C-18, 2.5 $\mu\text{m}$ , 4.6mm, solvent A 0.1 FA in water, solvent B 0.1%FA in ACN, t = 0, 10% B, t = 4 min, 95% B, t = 5.2 min, 10% B, t = 7 min, 10% B. The reaction mixture was filtered and washed with potassium iodide (2M, 25mL $\times$ 2 if used Hg(CN)<sub>2</sub>), saturated NaHCO<sub>3</sub> (10 mL $\times$ 2) and dried with sodium sulphate. The solvent was removed by vacuum to afford a light brown crude product. Then the crude product was purified by flash column (ethyl acetate/petroleum ether/methanol 12/4/1,) to afford a white powder (yield; see Table 1)

### 3.2 General method of glycosylation using DMF as solvent (Table 1, 5-12)

A mixture of novobiocin sodium (200 mg, 0.32 mmol), 1-Bromine-2,3,4,6-tetra-O-acetyl- $\alpha$ -D-glucose/galactose (0.132g, 0.32mmol), activated 4Å molecular sieves (approx. 1.5g) and anhydrous DMF (anhydrous, 20ml, or other solvent as in Table 1) were added into a 250 ml flask. Catalyst (eqv. shown in Table 1) was added into the reaction. The reaction mixture was stirred for 3 days under nitrogen gas protection at room temperature covered by foil and monitored by LC/MS. Water (120ml) was added into the system and the crude product was extracted by chloroform. The crude product was purified by flash column (ethyl acetate/petroleum ether/methanol 12/4/1, Rf = 0.3) to afford a white powder (yield; see Table 1).

### 3.3 General method of phase transfer glycosylation of novobiocin

Novobiocin (20mg 0.03mmol) was weighed and dissolved in a beaker that already contained 5 ml 0.15 M potassium carbonate water solution, stirred for 15 minutes.  $\alpha$ -1-Bromine-2,3,4,6-tetra-O-acetyl-D-glucose (53.9mg, 0.12mmol) and benzyltributylammonium bromide (11.4mg, 0.03mmol) were dissolved in 5ml chloroform. When the stirring of water-phase was complete, the colourless solution inside the beaker was transferred into the flask and little amount of 0.15M potassium carbonate water solution was used to wash the beaker. Once the separation of two layers was finished, the whole system was put into a 45°C oil bath (or room temperature) and stirred overnight. Organic layer was monitored by LC/MS. The water phase inside was removed and the organic phase was washed with saturated NaHCO<sub>3</sub> (20mL), brine (10mL) and deionized water (10mL $\times$  2). The brown organic phase was then concentrated under vacuum to obtain an orange solid residue and purified by Biotage reverse phase column (yield see Table2). Compound **6** was separated using prep-HPLC under linear gradient condition within 200 min. <sup>1</sup>HNMR (400 MHz, MeOD<sub>4</sub>):  $\delta$  = 7.66 (1 H, s, H3), 6.01 (1 H, d, J = 8.8 Hz, H6), 7.67 (1 H, d, J = 8.9 Hz, H7), 3.12 (2 H, m, H8), 5.10 (1 H, m, H9), 1.54 (6H, s, H11, H12), 7.63 (1 H, d, J = 8.8 Hz, H5'), 7.00 (1 H, d, J = 8.7 Hz, H6'), 2.11 (3 H, s, H11'), 5.36 (1 H, d, J = 1.88 Hz, H1''), 4.04 (1 H, m, H2''), 5.15 (1 H, dd, J = 3.18, 9.93 Hz, H3''), 3.40 (1 H, d, J = 12.6 Hz, H4''), 1.16 (3 H, s, H6''), 0.98 (3 H, s, H7''), 3.37 (3 H, s, H8''); 5.20 (1 H, d, J = 7.9 Hz, H1'''), 5.26 (1 H, m, H2'''), 5.30 (1 H, m, H3'''), 5.12 (1 H, m, H4'''), 4.18 (1 H, m, H5'''), 4.03 (2 H, m, 2 \* H6'''), 1.80-2.01 (12 H, s, 4\*CH<sub>3</sub>CO). <sup>13</sup>CNMR (125 MHz, MeOD<sub>4</sub>):  $\delta$  = 169.35 (C1), 158.35 (C2), 130.77 (C3), 125.03 (C4), 159.15 (C5), 110.41 (C6), 128.23 (C7), 29.16 (C8), 123.21 (C9), 134.0 (C10), 26.11(C11), 18.06 (C12), 163.16 (C2'), 108.84 (C3'), 164.52 (C4'), 124.00 (C5'), 115.09 (C6'), 158.15 (C7'), 114.46 (C8'), 153.33 (C9'), 118.18 (C10'), 6.56 (C11'), 99.91 (C1''), 71.07 (C2''), 73.20 (C3''), 82.85 (C4''), 79.83 (C5''), 29.08 (C6''), 23.34 (C7''), 61.87 (C8''), 167.30 (C9''), 99.84 (C1'''), 70.21 (C2'''), 68.85 (C3'''), 72.34 (C4'''), 72.34 (C5'''), 62.73 (C6'''), 20.58-20.83 (4\*CH<sub>3</sub>CO), 171.25-172.09 (4\*CH<sub>3</sub>CO). ESI<sup>+</sup> 811 [M+H]<sup>+</sup>, HRMS: 943.3360, calculated for C<sub>45</sub>H<sub>54</sub>N<sub>2</sub>O<sub>20</sub>: 943.3348.

### 3.4 DNA Gyrase Assay

The DNA gyrase assay was performed according to the supplier's procedure with novobiocin, glucosyl-novobiocin and galactosyl-novobiocin as inhibitors.

DNA gyrase (1U) was incubated with relaxed pBR322 (0.5µg) in a reaction volume of 30 µL at 37°C for 1 hr in TRIS-HCl (35mM, pH 7.5), KCl (24mM), MgCl<sub>2</sub> (4mM), DTT (2mM), spermidine (1.8mM), ATP (1mM), glycerol (6.5%, w/v) and albumin (0.1mg/mL).

After incubation the mixture was treated with equal volumes of chloroform /isoamyl alcohol (24:1) and the end point was determined by separating relaxed and super twisted plasmid pBR322 DNA by agarose gel electrophoresis (0.8% agarose with TBE buffer), running without ethidium bromide. After electrophoresis the gel was soaked in tank buffer containing ethidium bromide for one and half hours.

### 3.5 Docking studies and visualization

The crystal structure of the DNA gyrase (PDB id:1AJ6) was used as a receptor in docking studies. The ICM-Pro Molsoft software (Molsoft LLC, [www.molsoft.com](http://www.molsoft.com)) was used to carry out the docking studies. Ligand No. 1 (novobiocin) was extracted from crystal structure and redocked in the same position. The parameters used in obtaining the redocked conformation were used to dock other ligands. ICM ligedit module was used to generate ligand **2** and **3**. A binding pocket was defined by using ICM Finding Pocket tools. The docking grid was centred on the best pocket found, taking into account all residues around existing ligand (novobiocin). For each compound we used the APF superposition template method to obtain the most reliable results. The hydrogen bond analysis of obtained complexes was carried out with ICM Molsoft software.

### 3.6 Activity of novobiocin and novobiocin derivatives against *Escherichia coli*

The MICs of novobiocin and novobiocin derivatives were determined by micro-broth dilution technique in cation-adjusted Mueller-Hinton broth and with a bacterial inoculum of 5x10<sup>5</sup> CFU/ml. Microtitre plates were incubated aerobically at 37°C and read after 24h. The concentration range tested was 128 to 0.12µg/ml.

## 4. Conclusions

In conclusion, Koenigs-Knorr glycosylation gave only a thermodynamic controlled 4'-OH product. DMF proved to be the best condition, while other conventional catalysts were detrimental to the yield. Phase transfer reaction gave a kinetic controlled 5-OH product but still produced thermodynamic controlled 4'-OH, di-glycosylated glycosylation products and, possibly the more difficult 3''-OH glycosylation in the presence of a stronger base. This provides a powerful tool to generate various products. It also demonstrated that the galactose modification increased the antimicrobial activity



probably due to the enhanced uptake ability.

## Highlights

- Reaction conditions control glycosylation position.
- Glycosyl-novobiocin weaken the binding to DNA gyrase.
- Sugar moiety created new additional binding to the protein target.
- Galactosylation may enhance the activity against certain *E. coli* isolates.

## Keywords

Glycosylation; Novobiocin; Phase transfer reaction; Antibacterial activity.

## Acknowledgement

This work was supported by EPSRC (EP/K023071/1). We thank Dr Andreia M. R. Guimaraes for her technical help.

## Declaration

The authors declare no conflict of interest.

## Reference

- [1] J. Whitworth, in, 2013 <http://www.foodqualitynews.com/Industry-news/EFSA-infographic-The-fight-against-antimicrobial-resistance>.
- [2] A. Nakayama, A. Okano, Y. Feng, J.C. Collins, K.C. Collins, C.T. Walsh, D.L. Boger, *Org. Lett.*, 16 (2014) 3572-3575.
- [3] J.S. Thorson, T. Vogt, *Carbohydrate-Based Drug Discovery*, 2 (2003) 685-711.
- [4] S.A. Borisova, C. Zhang, H. Takahashi, H. Zhang, A.W. Wong, J.S. Thorson, H.-w. Liu, *Angewandte Chemie, International Edition*, 45 (2006) 2748-2753.
- [5] X. Fu, C. Albermann, C. Zhang, J.S. Thorson, *Organic Letters*, 7 (2005) 1513-1515.
- [6] C. Zhang, C. Albermann, X. Fu, J.S. Thorson, *Journal of the American Chemical Society*, 128 (2006) 16420-16421.
- [7] C. Zhang, B.R. Griffith, Q. Fu, C. Albermann, X. Fu, I.-K. Lee, L. Li, J.S. Thorson, *Science (Washington, DC, United States)*, 313 (2006) 1291-1294.
- [8] R.W. Gantt, P. Peltier-Pain, J.S. Thorson, *Nat. Prod. Rep.*, 28 (2011) 1811.
- [9] M. Yang, M.R. Proctor, D.N. Bolam, J.C. Errey, R.A. Field, H.J. Gilbert, B.G. Davis, *Journal of the American Chemical Society*, 127 (2005) 9336-9337.
- [10] X. Fu, C. Albermann, J. Jiang, J. Liao, C. Zhang, J.S. Thorson, *Nature Biotechnology*, 21 (2003) 1467-1469.
- [11] M. Trieb, C. Rauch, B. Wellenzohn, F. Wibowo, T. Loerting, E. Mayer, K.R. Liedl, *J. Biomol. Struct. Dyn.*, 21 (2004) 713-724.
- [12] F. Zunino, G. Pratesi, P. Perego, *Biochem. Pharmacol.*, 61 (2001) 933-938.
- [13] D. Kahne, C. Leimkuhler, L. Wei, C. Walsh, *Chem. Rev.*, 105 (2005) 425-448.
- [14] M. Gellert, M.H. O'Dea, T. Itoh, J.I. Tomizawa, *Proc. Nat. Acad. Sci. USA*, 73 (1976) 4474-4478.
- [15] M.G. Marcu, T.W. Schulte, L. Neckers, *J. Nat. Canc. Inst.*, 92 (2000) 242-248.
- [16] I.N. Cruz, Y. Zhang, M. de la Fuente, A. Schatzlein, M. Yang, *Anal. Biochem.*, 428 (2013) 107-109.
- [17] A. Maxwell, D.M. Lawson, *Curr. Top. Med. Chem.*, 3 (2003) 283-303.
- [18] A. Maxwell, *Mol. Microbiol.*, 9 (1993) 681-686.

- [19] D.C. Hooper, J.S. Wolfson, G.L. McHugh, M.B. Winters, M.N. Swartz, *Antimicrob. Agents Chemother.*, 22 (1982) 662-671.
- [20] C. Anderle, M. Stieger, M. Burrell, S. Reinelt, A. Maxwell, M. Page, L. Heide, *Antimicrob. Agents Chemother.*, 52 (2008) 1982-1990.
- [21] J.A. Burlison, C. Avila, G. Vielhauer, D.J. Lubbers, J. Holzbeierlein, B.S.J. Blagg, *J. Org. Chem.*, 73 (2008) 2130-2137.
- [22] U. Galm, S. Heller, S. Shapiro, M. Page, S.M. Li, L. Heide, *Antimicrob. Agents Chemother.*, 48 (2004) 1307-1312.
- [23] S.M. Patel, M. Fuente, S. Ke, A. Guimaraes, A.O. Oliyide, X. Ji, P. Stapleton, A. Osbourn, Y. Pan, D.J. Bowles, B.G. Davis, A. Schatzlein, M. Yang, *Chem. Commun.*, 47 (2011) 10569-10571.
- [24] J.A. Burlison, L. Neckers, A.B. Smith, A. Maxwell, B.S.J. Blagg, *Journal of the American Chemical Society*, 128 (2006) 15529-15536.
- [25] G.A. Holdgate, A. Tunnicliffe, W.H. Ward, S.A. Weston, G. Rosenbrock, P.T. Barth, I.W. Taylor, R.A. Pauptit, D. Timms, *Biochemistry*, 36 (1997) 9663-9673.
- [26] M. Yang, M.R. Proctor, D.N. Bolam, J.C. Errey, R.A. Field, H.J. Gilbert, B.G. Davis, *J. Am. Chem. Soc.*, 127 (2005) 9336-9337.