

# Efficient synthesis of glycosylated phenazine natural products and analogs with DISAL (methyl 3,5-dinitrosalicylate) glycosyl donors †

Jane B. Laursen,<sup>a,b</sup> Lars Petersen,<sup>b</sup> Knud J. Jensen<sup>\*a</sup> and John Nielsen<sup>\*a</sup>

<sup>a</sup> Department of Chemistry, The Royal Veterinary and Agricultural University, DK-1871 Frederiksberg C, Denmark. E-mail: [kjj@kvl.dk](mailto:kjj@kvl.dk); [jn@kvl.dk](mailto:jn@kvl.dk); Fax: +45 35 28 23 98; Tel: +45 35 28 24 28

<sup>b</sup> Department of Chemistry, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark

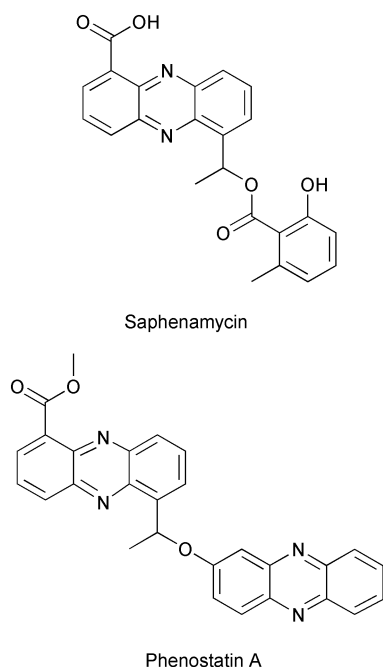
Received 18th June 2003, Accepted 5th August 2003

First published as an Advance Article on the web 15th August 2003

Inspired by the occurrence and function of phenazines in natural products, new glycosylated analogs were designed and synthesized. DISAL (methyl 3,5-dinitrosalicylate) glycosyl donors were used in an efficient and easily-handled glycosylation protocol compatible with combinatorial chemistry. Benzoylated D-glucose, D-galactose and L-quinovose DISAL glycosyl donors were synthesized in high yields and used under mild conditions to glycosylate methyl saphenate and 2-hydroxyphenazine. The glycosides were screened for biological activity and one compound showed inhibitory activity towards topoisomerase II.

## Introduction

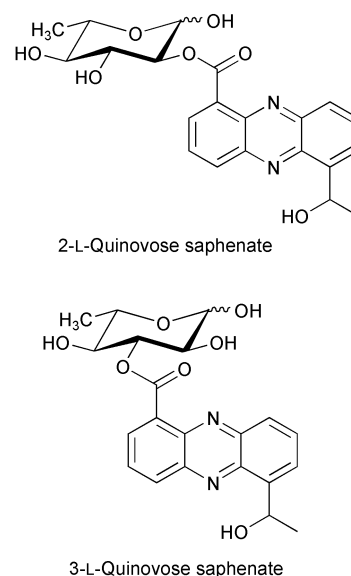
The need for new antibiotics and anticancer agents has inspired researchers to search beyond secondary metabolites produced by soil-borne bacteria and investigate the diverse pool of marine natural products to discover new pharmacologically relevant compounds.<sup>1</sup> Phenazine-containing antibiotics have been found not only in soil-derived species but also in marine microorganisms. Two such examples are saphenamycin, the 6-methylsalicylic acid ester of the natural secondary metabolite phenazine saphenic acid (6-(1-hydroxyethyl)phenazine-1-carboxylic acid),<sup>2</sup> and phenostatin A, the methyl ester of saphenic acid 2-hydroxyphenazine ether (Fig. 1), both isolated from



**Fig. 1** Structure of two phenazine containing natural products isolated from *Streptomyces* strains: saphenamycin and phenostatin A.

*Streptomyces*.<sup>3</sup> Our recent research on phenazine natural products include an improved synthetic route to saphenic acid,<sup>4</sup> the first chemical synthesis and absolute structure elucidation of saphenamycin,<sup>5</sup> as well as efficient solid-phase syntheses of highly potent antibiotic analogs of saphenamycin.<sup>6</sup> The physiological functions of these phenazines are still not fully understood, and proposed biological targets include DNA intercalation,<sup>7</sup> free-radical scavenging,<sup>8</sup> and inhibition of topoisomerase I or II.<sup>9</sup>

During the last decade, glycoconjugates like vancomycin,<sup>10</sup> doxorubicin,<sup>11</sup> epipodophyllotoxin<sup>12</sup> and aminoglycoside antibiotics<sup>13</sup> have attracted much attention in the design and development of drugs, which target microbial and viral infections (HIV) or cancer. Interestingly, glycosylated saphenic acid analogs with antibacterial activity against a broad range of Gram-negative and Gram-positive bacteria were isolated in the early 1990's from a *Streptomyces* strain.<sup>14</sup> The derivatives were shown to be mixtures of  $\alpha$ - and  $\beta$ -anomers of the rare monosaccharide L-quinovose (6-deoxy-L-glucose) linked at O-2 or O-3 to saphenic acid through a labile ester bond (Fig. 2).



**Fig. 2** Structure of the carbohydrate containing antibiotics, saphenic acid L-quinovose esters.

† Electronic supplementary information (ESI) available: HPLC analysis data for compounds 2, 3 and 6–20. See <http://www.rsc.org/suppdata/ob/b3/b306789k/>

Classical glycosylation methods require general or specific Lewis acid activation of the glycosyl donor. We have developed an efficient method for glycosylation under strictly neutral, mildly basic, or very mild, acidic conditions, as described in recent papers.<sup>15,16</sup> In this glycosylation technique, the anomeric leaving group on the donor is methyl 3,5-dinitrosalicylate (DISAL), which combines high reactivity with stability on prolonged storage. Preceding reports on DISAL glycosylation show excellent yields in the glycosylation of simple alcohols and good yields for more complex carbohydrate acceptors. The potential of DISAL glycosyl donors has previously been demonstrated for solid-phase oligosaccharide synthesis,<sup>15b</sup> intramolecular glycosylations,<sup>15c</sup> and for the synthesis of hexasaccharides.<sup>15d</sup>

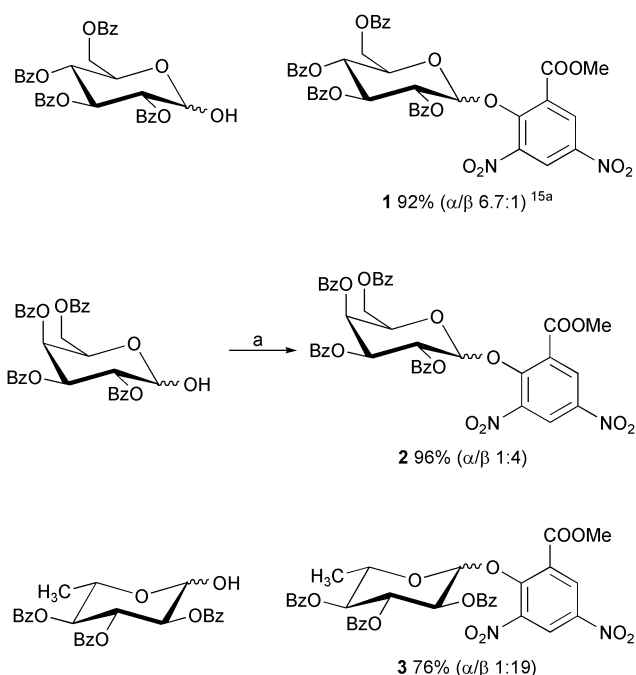
Our research aims at combining the structural input from natural products with new efficient methodologies for high-throughput synthesis and combinatorial chemistry to facilitate access to new biologically active natural product analogs.<sup>17</sup> Stable analogs of glycophenazines, in which the labile ester linkage had been substituted for a configurationally stable glycoside bond, could be accessed by combining our novel glycosylation protocol with our chemistry for the synthesis of saphenic acid for the preparation of novel phenazine compounds. Glycosylation with DISAL donors could provide us with stable and anomeric well-defined derivatives of 2-hydroxyphenazine and saphenic acid, which would be the first members of a hitherto unexplored class of natural product analogs.

As phenazine derivatives are not stable to hydrogenolysis conditions,<sup>18</sup> this precluded the use of benzyl protecting groups on the glycosyl donor. However, benzoyl protecting groups can be removed by treatment in methanol with catalytic amounts of methoxide. Benzoyl protected DISAL glycosides become efficient glycosyl donors in the presence of LiClO<sub>4</sub>.<sup>15b</sup> These very mild conditions should be compatible with polycyclic heteroaromatics such as phenazine derivatives. Deprotection of the phenazine glycosides would be performed in two steps, giving rise to two sets of compounds, both with potential biologically interesting properties, first the glycoside methyl esters and then the corresponding free acids.

## Results and discussion

The DISAL glycosyl donors were prepared by convenient nucleophilic aromatic substitution of an activated aryl fluoride in the presence of an insoluble inorganic base, Li<sub>2</sub>CO<sub>3</sub> and a soluble organic base. Earlier studies have shown that use of 4-*N,N*-dimethylaminopyridine (DMAP) as the soluble base provided DISAL  $\alpha$ -glucopyranosides with high selectivity, whereas 1,4-dimethylpiperazine (DMP) gave predominantly  $\beta$ -glycosides.<sup>15a</sup> Here, perbenzoylated gluco-, galacto- and quinovopyranoses were converted into their corresponding DISAL glycosyl donors by DMAP catalyzed reaction with methyl 2-fluoro-3,5-dinitrobenzoate in very good to excellent yields (Scheme 1, 92%, 96% and 76% for **1**, **2** and **3**, respectively) but predominantly as DISAL  $\beta$ -galacto- and quinovopyranosides. As discussed in a previous paper, the anomer distribution of the donors is determined by the nucleophilicity and rate of interconversion of the individual anomeric alkoxides.<sup>15a</sup>

Glycosyl acceptor methyl saphenate (**4**) was synthesized by activation of racemic saphenic acid with oxalyl chloride and subsequent treatment with methanol. Racemic saphenic acid was available to us through a synthesis in a few steps from readily available starting materials, as previously reported.<sup>4</sup> 2-Hydroxyphenazine (**5**) was synthesized *via* the Beirut reaction in two steps from hydroquinone and benzofurazan oxide.<sup>19</sup> Racemic methyl saphenate (**4**) and 2-hydroxyphenazine (**5**) were both glycosylated with glycosyl donors **1–3** giving rise to six protected carbohydrate phenazine derivatives **6–11** in good yields (Scheme 2). In previous DISAL studies we established



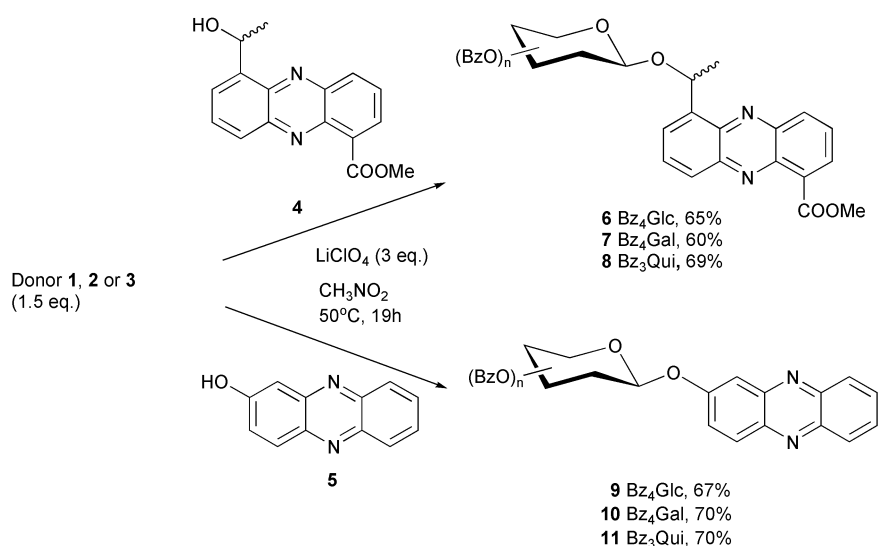
**Scheme 1** Synthesis of glycosyl donors **1** (as recently reported<sup>15a</sup>), **2**, and **3**. a) Li<sub>2</sub>CO<sub>3</sub>, methyl 2-fluoro-3,5-dinitrobenzoate, DMAP, CH<sub>2</sub>Cl<sub>2</sub>.

three sets of standard conditions for glycosylation with DISAL donors (1.5 equiv. to 3 equiv.): (i) donor and acceptor in NMP at 40–60 °C; (ii) donor and acceptor in (CH<sub>2</sub>Cl)<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub> or CH<sub>3</sub>NO<sub>2</sub> in the presence of LiClO<sub>4</sub> (2.0–3.7 equiv.), in some cases also with Li<sub>2</sub>CO<sub>3</sub> as an acid scavenger (2 equiv.); (iii) donor and acceptor in (CH<sub>2</sub>Cl)<sub>2</sub> or CH<sub>2</sub>Cl<sub>2</sub> in the presence of LiClO<sub>4</sub> and Bu<sub>4</sub>Nl.<sup>15</sup> Conditions for the glycosylation reactions described herein were adapted from these earlier studies. Activation of the benzoylated glycosyl donors **1–3** with the very mild Lewis acid LiClO<sub>4</sub> (4 equiv.) proved efficient in all cases and afforded products **6–11** in 60–70% yield as pure  $\beta$ -anomers, indicating neighboring group participation by the 2-*O*-benzoyl group (Scheme 2).<sup>20</sup> However, glycosides **6–8** were diastereomeric mixtures due to the use of racemic saphenic acid. The glycosylation reactions were carried out in standard plastic vials, followed by analytical HPLC and full conversion was observed within 19 h for all glycosylations. The phenolic glycosyl acceptor **5** was glycosylated somewhat faster than the secondary benzylic alcohol in **4**. No large reactivity differences were observed for the three donors **1–3**. Purification of the glycosylation products proceeded smoothly by direct injection of the filtered reaction mixtures into a preparative HPLC followed by lyophilization of fractions without additional work-up. Incidentally, this is also the first report on glycosylation of phenols by DISAL donors.

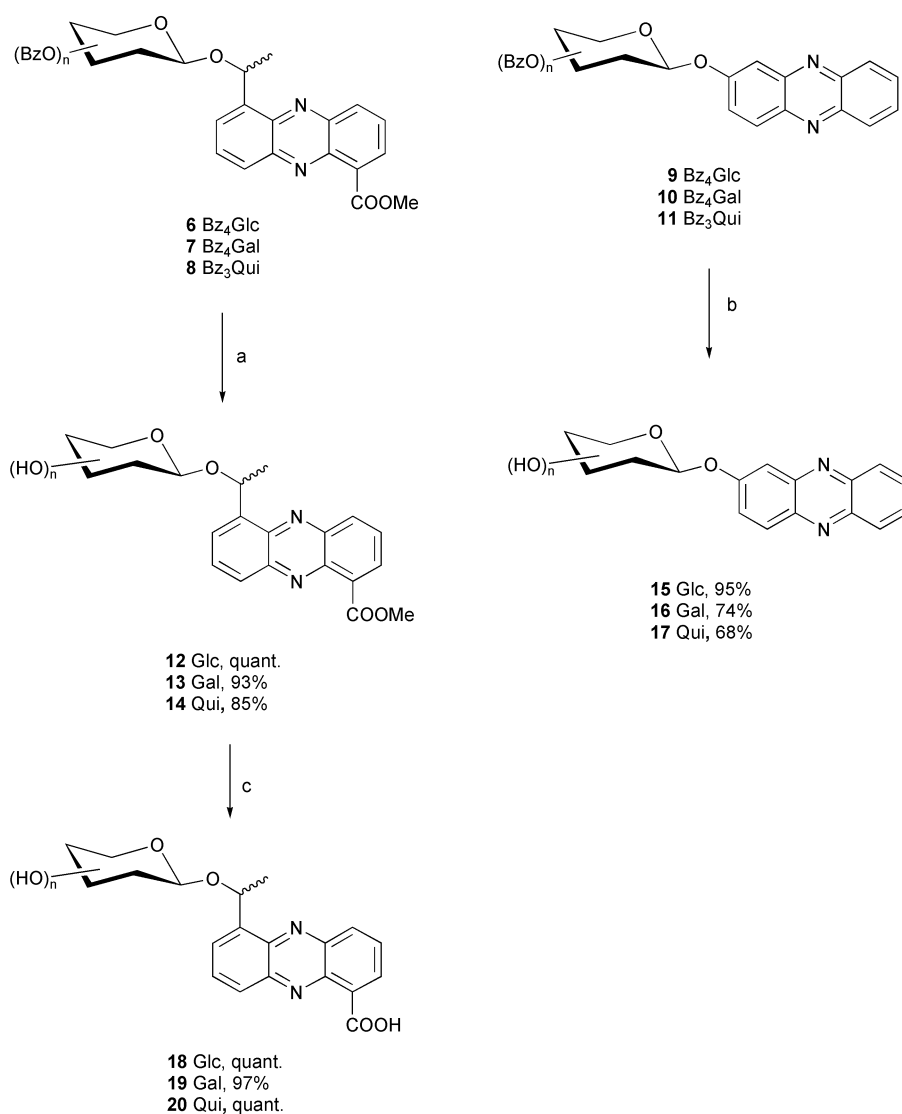
The glycosides were deprotected in two steps. First, the protected glycosides **6–11** were subjected to Zemplén debenzoylation, which afforded compounds **12–17** (Scheme 3) in near-quantitative yields, except for 2-hydroxyphenazine derivatives **10** and **11**, which were only sparsely soluble and less reactive. Debenzoylation of these proceeded in only moderate yields. Then, the free acids were liberated by hydrolysis of the methyl esters **12–14** with LiOH in near-quantitative yields affording acids **18–20** (Scheme 3).

This ensemble of 3 by 3 compounds (**12–20**) was tested for antibiotic activity against a range of Gram-negative bacteria that were known to be sensitive to saphenamycin.<sup>21</sup> However, none of the tested glycosides, all of  $\beta$ -configuration, exhibited any inhibition of bacterial growth.

Glycosides **12–20** were also screened for topoisomerase II $\alpha$  inhibition in a decatenation assay, since several known phenazine-containing compounds have shown antitumor



**Scheme 2** Glycosylation reactions.



**Scheme 3** Deprotection scheme. a) Anhydr. MeOH, NaOMe (cat.). b) Anhydr. MeOH, min. amount DMF, NaOMe (cat.). c) LiOH·H<sub>2</sub>O, MeOH-H<sub>2</sub>O (3 : 1).

effects.<sup>2</sup> Topoisomerase II $\alpha$  is an enzyme responsible for topological changes in DNA (*e.g.* decatenation and relaxation of supercoiling), which is largely confined to proliferating cells, and therefore is an obvious target for anticancer drugs. Some antitumor agents trap the enzyme by inhibiting the formation of the cleavable complex (catalytic inhibitors), thus no decaten-

ation will take place.<sup>22</sup> Other antitumor drugs are cytotoxic or poisonous due to stabilization of the cleavable covalent DNA-topoisomerase II intermediate, hence inhibiting the DNA-religation step. Decatenation assays may be complemented by damage control assays in order to distinguish between topoisomerase poisons and catalytic inhibitors. Glyco-

side **20** reduced decatenation by *ca.* 50% (relative to dextrazoxane) at concentrations above 150  $\mu\text{M}$ . In the damage control assay 100% survival of cells was observed at concentrations up to 300  $\mu\text{M}$  showing that glycoside **20** does not act as a cell poison. A catalytic inhibitor will protect the cell against the cytotoxic effect of a poison like daunorubicin or etoposide, hence poison-induced cell death will decrease. In the presence of epotoposide and daunorubicin compound **20** induced increased cell growth at concentrations of 10–100  $\mu\text{M}$  and some inhibition of poison induced cell death was observed at a concentration of 300  $\mu\text{M}$ .

## Conclusion

We have applied novel DISAL glycosyl donors in the synthesis of glycosylated phenazines in an experimentally simple and high-yielding protocol well-suited for combinatorial chemistry. DISAL glycosyl donors were synthesized in good yields based on benzoylated D-glucose, D-galactose, and the rare carbohydrate L-quinovose and proved stable on prolonged storage at 5 °C. The DISAL glycosides became efficient glycosyl donors under very mild conditions ( $\text{LiClO}_4$  in nitromethane) and were easy to handle both during reaction, work-up and purification (direct injection of reaction mixtures into preparative HPLC). Phenazine glycosides were obtained in good yields as pure  $\beta$ -anomers as was anticipated because of the anchimeric assistance provided by the 2-*O*-benzoyl group. Zemplén deacylation removed the benzoyl protecting groups in near-quantitative yields, though two rather sparsely soluble compounds gave only moderate yields. Final methyl ester hydrolysis proceeded quantitatively yielding the free acids. The nine glycosylated phenazine derivatives were tested for antibiotic activity and topoisomerase II inhibition. While no antibiotic activity was detected in any of the glycosides, quinovosyl derivative **20** showed catalytic inhibition of the topoisomerase II $\alpha$  at 100  $\mu\text{M}$  and no cytotoxicity at concentrations up to 300  $\mu\text{M}$ .

## Experimental

### General procedures

Commercially available reagents were used without further purification unless otherwise noted. Racemic saphenic acid was prepared as previously reported.<sup>4</sup> Solvents were distilled prior to use and stored over molecular sieves. VLC was performed using Merck 60H silica gel. Flash chromatography was performed automatically on a Biotage Quad3+ apparatus with pre-packed columns (KP-SIL, 32–63  $\mu\text{m}$ , 60 Å). Corrected melting points were measured in open capillary tubes. Preparative HPLC was performed on a Waters 600 system with a Waters 996 diode array detector and three consecutive columns (40  $\times$  100 mm prep. NOVA Pak HR C18 6  $\mu\text{m}$ , 60 Å). Linear gradients of  $\text{CH}_3\text{CN}$  and water (MilliQ) were used.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, gHSQC and H,H-COSY spectra were recorded on a Varian Mercury 300 or a Varian Unity Inova 500 spectrometer. The chemical shifts are referenced to an internal standard. Accurate mass determination (high resolution MS, HR-MS) was performed on a Micromass LCT apparatus with an AP-ESI probe. The purity of compounds **2–3** and **6–20** was verified by analytical HPLC analysis using a Waters system (600 control unit, 996 photodiode array (PDA) detector, 717 Plus autosampler) with a Waters X-Terra C18 column (3.0  $\times$  50 mm, 3.8  $\mu\text{m}$ ) using linear gradients of  $\text{CH}_3\text{CN}$  and water (MilliQ) with 0.1% TFA if needed.

### General procedure for the synthesis of glycosyl donors **1**, **2** and **3**

In a dried flask, protected carbohydrate derivatives (2,3,4,6-tetra-*O*-benzoyl-D-glucopyranose, 2,3,4,6-tetra-*O*-benzoyl-D-galactopyranose or 6-deoxy-2,3,4-tri-*O*-benzoyl-L-glucopyran-

ose),  $\text{Li}_2\text{CO}_3$  (2 equiv.), and methyl 2-fluoro-3,5-dinitrobenzoic ester (1.2 equiv.) were suspended in  $\text{CH}_2\text{Cl}_2$  (1–2 mL  $\text{mmol}^{-1}$ ). Reactions proceeded in the presence of DMP or DMAP. (1) For reactions with 1,4-dimethylpiperazine catalysis, DMP (0.5 equiv.) was added to the suspension and the mixture was stirred for 2–3 h. (2) For reactions with DMAP catalysis, DMAP (0.3 equiv.) was dissolved in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) and added to the suspension in 5 portions over 20 min. After addition of the first portion, the color changed from slightly discolored to a strong yellow–brown. After stirring for an additional 10 min, the products were isolated by VLC chromatography (eluting with toluene–ethyl acetate 1 : 0  $\rightarrow$  10 : 1).

**2,4-Dinitro-6-(methoxycarbonyl)phenyl 2,3,4,6-tetra-*O*-benzoyl- $\alpha$ -D-glucopyranoside (**1a**).** 92% yield,  $\alpha$  :  $\beta$  6.7 : 1. The physical and analytical data were in agreement with previous reports.<sup>15a</sup> Electrospray HR-MS (positive mode): *m/z* calcd. for  $\text{C}_{42}\text{H}_{36}\text{N}_3\text{O}_{16}$  ( $\text{M} + \text{NH}_4$ )<sup>+</sup> 838.2034, found 838.2103 (25%); *m/z* calcd. for  $\text{C}_{42}\text{H}_{32}\text{N}_3\text{NaO}_{16}$  ( $\text{M} + \text{Na}$ )<sup>+</sup> 843.1650, found 843.1619 (50%); *m/z* calcd. for  $\text{C}_{42}\text{H}_{32}\text{N}_2\text{KO}_{16}$  ( $\text{M} + \text{K}$ )<sup>+</sup> 859.1389, found 859.1387 (10%), *m/z* calcd. for  $\text{C}_{27}\text{H}_{23}\text{O}_8$  ( $\text{M} - \text{DISAL} - \text{BzOH} + \text{H}$ )<sup>+</sup> 475.1393, found 475.1373 (100%).

**2,4-Dinitro-6-(methoxycarbonyl)phenyl 2,3,4,6-tetra-*O*-benzoyl- $\beta$ -D-galactopyranoside (**2b**).** 96% yield,  $\alpha$  :  $\beta$  1 : 4, mp 88–91 °C. Anal. HPLC (215 nm) >95%.  $^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.72 (1H, d,  $J = 3.0$  Hz), 8.69 (1H, d,  $J = 3.0$  Hz), 8.22–7.14 (20H, m), 6.14 (1H, dd,  $J = 7.7$  and 10.7 Hz, H-2), 5.99 (1H, br d,  $J = 3.4$  Hz, H-4), 5.71 (1H, d,  $J = 7.7$  Hz, H-1 $\beta$ ), 5.68 (1H, dd,  $J = 3.4$  and 10.7 Hz, H-3), 4.51 (1H, dd,  $J = 7.0$  and 11.1 Hz, H-6), 4.34 (1H, dd,  $J = 6.0$  and 11.1 Hz, H-6'), 4.30 (1H, br dd,  $J = 6$  and 7 Hz, H-5), 3.81 (3H, s, OMe).  $^{13}\text{C}$ -NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  165.9, 165.6, 165.55, 165.4, 162.9, 150.8, 147.0, 143.7, 134.0, 133.6, 130.9, 130.5–128.4 (m, 22C), 125.5, 123.0, 102.4 (C-1 $\beta$ ), 72.5, 71.5, 70.0, 67.7, 61.6, 53.4 (OMe). Electrospray HR-MS (positive mode): *m/z* calcd. for  $\text{C}_{42}\text{H}_{36}\text{N}_3\text{O}_{16}$  ( $\text{M} + \text{NH}_4$ )<sup>+</sup> 838.2096, found 838.2103 (25%); *m/z* calcd. for  $\text{C}_{42}\text{H}_{32}\text{N}_2\text{NaO}_{16}$  ( $\text{M} + \text{Na}$ )<sup>+</sup> 843.1650, found 843.1637 (70%); *m/z* calcd. for  $\text{C}_{42}\text{H}_{34}\text{N}_2\text{NaO}_{17}$  ( $\text{M} + \text{Na} + \text{H}_2\text{O}$ )<sup>+</sup> 861.1755, found 861.2634 (100%); *m/z* calcd. for  $\text{C}_{27}\text{H}_{23}\text{O}_8$  ( $\text{M} - \text{DISAL} - \text{BzOH} + \text{H}$ )<sup>+</sup> 475.1393, found 475.1380 (45%).

**2,4-Dinitro-6-(methoxycarbonyl)phenyl 2,3,4-tri-*O*-benzoyl-6-deoxy- $\beta$ -L-glucopyranoside (**3b**).** 76% yield,  $\alpha$  :  $\beta$  1 : 19, mp 81–83 °C. Anal. HPLC (215 nm) 94%.  $^1\text{H}$ -NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.79 (1H, d,  $J = 3$  Hz), 8.72 (1H, d,  $J = 3$  Hz), 8.2–7.1 (15H, m), 5.9 (1H, t,  $J = 9.9$  Hz, H-4), 5.8 (1H, dd,  $J = 7.5$  and 9.7 Hz, H-2), 5.59 (1H, d,  $J = 7.5$  Hz, H-1 $\beta$ ), 5.39 (1H, t,  $J = 9.6$  Hz, H-3), 3.83 (3H, s, OMe), 3.81 (1H, m, H-5), 1.26 (3H, d,  $J = 9.3$  Hz, H-6).  $^{13}\text{C}$ -NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.0, 165.8, 165.7, 163.8, 150.8, 146.2, 144.1, 133.7, 133.6, 133.7, 130.3–128.5 (m, 17C), 123.0, 102.2 (C-1 $\beta$ ), 73.5, 72.4, 72.3, 71.9, 53.9 (OMe), 19.3 (C-6). Electrospray HR-MS (positive mode): *m/z* calcd. for  $\text{C}_{35}\text{H}_{32}\text{N}_3\text{O}_{14}$  ( $\text{M} + \text{NH}_4$ )<sup>+</sup> 718.1884, found 718.1858 (50%); *m/z* calcd. for  $\text{C}_{35}\text{H}_{28}\text{N}_2\text{NaO}_{14}$  ( $\text{M} + \text{Na}$ )<sup>+</sup> 723.1438, found 723.1437 (15%); *m/z* calcd. for  $\text{C}_{27}\text{H}_{23}\text{O}_7$  ( $\text{M} - \text{DISAL}$ )<sup>+</sup> 459.1393, found 459.1380 (100%).

### General procedure for the glycosylation reactions and purification

Glycosyl donor (**1**, **2** or **3**; 0.12 mmol, 1.5 equiv.), glycosyl acceptor (**4** or **5**; 0.10 mmol, 1 equiv.),  $\text{LiClO}_4$  (0.4 mmol, 3 equiv.) and crushed 4 Å molecular sieves (50 mg) and a magnet were placed in a 5 mL plastic tube with a septum. The tube was dried under high vacuum for 1 h, filled with argon,  $\text{CH}_3\text{NO}_2$  (0.7 mL) was added and the reaction mixture was stirred for 0.5 h at room temperature. The temperature was raised to 50 °C and stirring was continued for 19 h. The reaction mixture was filtered (through cotton wool and a microfilter) and loaded onto a preparative HPLC column with  $\text{CH}_3\text{CN}$  or

1 : 1 DMF-CH<sub>3</sub>CN (if necessary for dissolution). Purification on preparative HPLC (C18 column, 10% to 95% CH<sub>3</sub>CN over 65 min) furnished the glycosylation products **6–11**.

**Methyl 6-[(R/S)-1-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)ethyl]phenazine-1-carboxylic ester (6)**. 65% yield, pure β, 1 : 1 mixture of diastereomers (β-glycosides of both enantiomers of saphenic acid), mp 213–214 °C. Anal. HPLC (215 nm) >95%. <sup>1</sup>H-NMR for pairs of diastereomers (500 MHz, CDCl<sub>3</sub>) δ 8.27 and 8.26 (1H, dd, *J* = 1.3 and 5.5 Hz, H-4'), 8.22 and 8.20 (1H, dd, *J* = 1.3 and 7.2 Hz, H-2'), 8.15 and 8.11 (1H, dd, *J* = 1.3 and 8.6 Hz, H-9'), 8.08 (1H, dd, *J* = 1.3 and 8.2 Hz, H-7'), 8.05–8.00 (2H, m, H-3' and H-8'), 7.92–7.15 (20H, m), 6.38 and 6.24 (1H, q, *J* = 6.4 Hz, H-1"), 5.94 and 5.78 (1H, t, *J* = 9.6 Hz, H-3), 5.73–5.63 (2H, m, H-2 and H-4), 5.22 and 4.94 (1H, d, *J* = 7.7 Hz, H-1β), 4.71 and 4.41 (1H, dd, *J* = 3.4 and 11.9 Hz, H-6), 4.57 and 4.36 (1H, dd, *J* = 5.6 and 11.9 Hz, H-6'), 4.1 (4H, m, H-5 and OMe), 1.58 and 1.42 (3H, d, *J* = 6.4 Hz, H-2'). <sup>13</sup>C-NMR for pairs of diastereomers (75 MHz, CDCl<sub>3</sub>) δ 167.4, 166.0, 165.9, 165.3, 165.1, 143.5, 142.1, 141.9, 141.6, 140.8, 134.9–127.7 (m, 31C), 100.5 and 100.3 (C-1β), 73.7, 73.2 and 73.1, 72.5 and 72.4, 72.3 and 70.2, 65.2 and 65.1, 63.6 and 63.4 (C-1'), 52.9 (OMe), 23.9 and 23.0 (C-2'). Electrospray HR-MS (positive mode): *m/z* calcd. for C<sub>50</sub>H<sub>41</sub>N<sub>2</sub>O<sub>12</sub> (M + H)<sup>+</sup> 861.2660, found 861.2503 (100%).

**Methyl 6-[(R/S)-1-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)ethyl]phenazine-1-carboxylic ester (7)**. 60% yield, pure β, 1 : 1 mixture of diastereomers (β-glycosides of both enantiomers of saphenic acid), mp 215–216 °C. Anal. HPLC (215 nm) >95%. <sup>1</sup>H-NMR for pairs of diastereomers (300 MHz, CDCl<sub>3</sub>) δ 8.17 and 8.17 (1H, dd, *J* = 1.2 and 5.7 Hz, H-4'), 8.10 and 8.08 (1H, dd, *J* = 1.5 and 6.0 Hz, H-2'), 8.04 and 8.02 (1H, dd, *J* = 1.2 and 8.1 Hz, H-9'), 8.01–7.87 (3H, m, H-3', H-7' and H-8'), 7.73–7.06 (20H, m), 6.28 and 6.19 (1H, q, *J* = 6.6 Hz, H-1"), 5.87–5.77 (2H, m, H-2 and H-4), 5.53 and 5.34 (1H, dd, *J* = 3.6 and 10.5 Hz, H-3), 5.06 and 4.80 (1H, d, *J* = 8.1 Hz, H-1β), 4.66 and 4.35 (1H, dd, *J* = 6.6 and 11.4 Hz, H-6), 4.40–4.12 (2H, m, H-5, H-6'), 3.98 (3H, s, OMe), 1.62 and 1.44 (3H, d, *J* = 6.6 Hz, H-2'). <sup>13</sup>C-NMR for pairs of diastereomers (75 MHz, CDCl<sub>3</sub>) δ 167.4 and 167.3, 166.1 and 165.9, 165.81 and 165.78, 165.7 and 165.6, 165.5, 143.6 and 143.5, 142.0, 141.9, 141.3, 140.8, 134.0–128.4 (m, 31C), 102.4 and 100.7 (C-1β), 73.5 and 72.5, 72.04 and 71.99, 71.7 and 71.6, 70.4 and 70.2, 68.5 and 68.4, 62.3 and 62.2 (C-1'), 52.9 (OMe), 22.9 and 21.8 (C-2'). Electrospray HR-MS (positive mode): *m/z* calcd. for C<sub>50</sub>H<sub>41</sub>N<sub>2</sub>O<sub>12</sub> (M + H)<sup>+</sup> 861.2660, found 861.2634 (100%); *m/z* calcd. for C<sub>50</sub>H<sub>40</sub>N<sub>2</sub>NaO<sub>12</sub> (M + Na)<sup>+</sup> 883.2574, found 883.2484 (5%).

**Methyl 6-[(R/S)-1-(2,3,4-tri-O-benzoyl-β-L-glucopyranosyl)ethyl]phenazine-1-carboxylic ester (8)**. 69% yield, pure β, 1 : 1 mixture of diastereomers (β-glycosides of both enantiomers of saphenic acid), mp 169–170 °C. Anal. HPLC (215 nm) >95%. <sup>1</sup>H-NMR for pairs of diastereomers (300 MHz, CDCl<sub>3</sub>) δ 8.45 and 8.35 (1H, dd, *J* = 1.2 and 8.7 Hz, H-4'), 8.25–8.21 (1H, m, H-2'), 8.20 and 8.10 (1H, dd, *J* = 0.9 and 8.7 Hz, H-9'), 8.04 and 8.02 (1H, *J* = 0.9 and 7.5 Hz, H-7'), 7.92–7.15 (17H, m, H-3' and H-8', Ar-H), 6.35 and 6.26 (1H, q, *J* = 6.6 Hz, H-1"), 5.86 and 5.69 (1H, t, *J* = 9.8 Hz, H-4), 5.66–5.59 (1H, m, H-2), 5.37 and 5.31 (1H, t, *J* = 9.3 Hz, H-3), 5.17 and 4.83 (1H, d, *J* = 8.1 Hz, H-1β), 4.11 and 4.10 (3H, s, OMe), 3.77 (1H, m, H-5), 1.71 and 1.57 (3H, d, *J* = 6.3 Hz, H-2'), 1.43 and 1.12 (3H, d, *J* = 6.3 Hz, H-6). <sup>13</sup>C-NMR for pairs of diastereomers (75 MHz, CDCl<sub>3</sub>) δ 166.1, 166.0, 165.6, 165.4, 142.1, 141.9, 141.8, 141.6, 140.8, 133.9–127.8 (m, 25C), 100.1 (C-1β), 74.3 and 74.2, 73.32 and 73.30, 72.9 and 72.8, 70.83 and 70.82, 66.4 and 66.0 (C-1'), 52.9 (OMe), 24.1 and 22.8 (C-2'), 17.9 and 17.7 (C-6). Electrospray HR-MS (positive mode): *m/z*

calcd. for C<sub>43</sub>H<sub>37</sub>N<sub>2</sub>O<sub>10</sub> (M + H)<sup>+</sup> 741.2448, found 741.2305 (100%).

**2-(2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl)phenazine (9)**. 67% yield, mp 195–197 °C. Anal. HPLC (215 nm) >95%. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ 8.23 (1H, d, *J* = 8.5 Hz, H-6'), 8.16 (1H, d, *J* = 8.5 Hz, H-9'), 8.10 (1H, d, *J* = 9.4 Hz, H-4'), 8.06–7.78 (10H, m, 8Ar-H, H-7', H-8'), 7.76 (1H, d, *J* = 2.6 Hz, H-1'), 7.57–7.24 (13H, m, 12Ar-H, H-3'), 6.08 (1H, dd, *J* = 9.0 and 9.4 Hz, H-3), 5.95 (1H, dd, *J* = 8.1 and 8.5 Hz, H-2), 5.82 (1H, t, *J* = 9.4 Hz, H-4), 5.79 (1H, d, *J* = 8.1 Hz, H-1β), 4.77 (1H, dd, *J* = 2.1 and 12.0 Hz, H-6), 4.58 (1H, dd, *J* = 6.4 and 12.0 Hz, H-6'), 4.52 (1H, m, H-5). <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) δ 166.26, 165.92, 165.39, 165.26, 158.01, 144.54, 143.72, 142.73, 141.19, 133.80, 133.66, 133.59, 133.20, 131.42, 130.86, 130.1–128.5 (m, 23C), 125.96, 109.74 (C-1'), 98.80 (C-1β), 73.30, 72.92, 71.76, 69.55, 63.33. Electrospray HR-MS (positive mode): *m/z* calcd. for C<sub>46</sub>H<sub>35</sub>N<sub>2</sub>O<sub>10</sub> (M + H)<sup>+</sup> 775.2292, found 775.2300 (100%).

**2-(2,3,4,6-Tetra-O-benzoyl-β-D-galactopyranosyl)phenazine (10)**. 70% yield, mp 138 °C. Anal. HPLC (215 nm) >95%. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ 8.23 (1H, d, *J* = 8.5 Hz, H-6'), 8.20–8.08 (3H, m, 2Ar-H, H-9'), 7.97 (1H, d, *J* = 9.4 Hz, H-4'), 7.95–7.78 (8H, m, 6Ar-H, H-7', H-8'), 7.68–7.20 (14H, m, 12Ar-H, H-1', H-3'), 6.18 (1H, dd, *J* = 7.8 and 10.2 Hz, H-2), 6.10 (1H, m, H-3), 5.85–5.72 (2H, m, H-1β, H-4), 4.58–4.53 (3H, m, H-5, 2H-6). <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) δ 166.2, 165.7, 165.6, 165.4, 158.1, 144.5, 143.7, 136.6, 136.2, 136.1, 133.9, 133.7, 133.6, 133.4, 131.4, 130.9, 130.3–126.0 (m, 23C), 109.7 (C-1'), 99.3 (C-1β), 72.5, 71.8, 69.5, 68.2, 62.5. Electrospray HR-MS (positive mode): *m/z* calcd. for C<sub>46</sub>H<sub>35</sub>N<sub>2</sub>O<sub>10</sub> (M + H)<sup>+</sup> 775.2292, found 775.2277 (100%).

**2-(2,3,4,6-Tri-O-benzoyl-6-deoxy-β-L-glucopyranosyl)phenazine (11)**. 70% yield, mp 136 °C. Anal. HPLC (215 nm) >95%. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ 8.24–8.21 (2H, m, H-6' and H-9'), 8.12 (1H, d, *J* = 9.0 Hz, H-4'), 7.99–7.74 (9H, m, H-1', H-7', 7Ar-H), 7.56–7.26 (10H, m, H-3', H-8', 8Ar-H), 5.98 (1H, t, *J* = 9.0 Hz, H-4), 5.86 (1H, dd, *J* = 8.0 and 10.0 Hz, H-2), 5.71 (1H, d, *J* = 8.0 Hz, H-1β), 5.50 (1H, t, *J* = 9.5 Hz, H-3), 4.21 (1H, m, H-5), 1.50 (3H, d, *J* = 6.0 Hz, H-6). <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) δ 166.4, 166.0, 165.7, 158.4, 142.7, 133.7, 133.5, 133.4, 131.4–126.3 (m, 23C), 125.3 (C-3'), 114.4 (C-1'), 98.8 (C-1β), 73.7, 73.0, 72.1, 71.6, 17.9 (C-6). Electrospray HR-MS (positive mode): *m/z* calcd. for C<sub>39</sub>H<sub>31</sub>N<sub>2</sub>O<sub>8</sub> (M + H)<sup>+</sup> 655.2080, found 655.2073 (100%).

#### General procedure for Zemplén debenzoylations

The benzoyl protected glycoside (**6–11**) was dissolved in dry MeOH. For complete dissolution of **10**, a minimum amount of DMF was added. A catalytic amount of NaOMe was added and the reaction was stirred at room temperature for 19 h. In the synthesis of **15–17** the temperature was raised to 60 °C, and stirring was continued for 1–4 h in order to obtain complete conversion of **9–11**. On cooling to room temperature, the reaction mixture was acidified to pH 6 with AcOH (conc.), then evaporated to dryness and dried under high vacuum. Purification on VLC or Biotage (eluting with CH<sub>2</sub>Cl<sub>2</sub>-MeOH 90 : 10 or 80 : 20) and evaporation or crystallization from Et<sub>2</sub>O gave deprotected compounds **12–17**.

**Methyl 6-[(R/S)-1-(β-D-glucopyranosyl)ethyl]phenazine-1-carboxylic ester (12)**. Yield quant., pure β, 1 : 1 mixture of diastereomers (β-glycosides of both enantiomers of saphenic acid), mp 179–180 °C. Anal. HPLC (215 nm) >95%. <sup>1</sup>H-NMR for pairs of diastereomers (300 MHz, CD<sub>3</sub>OD) δ 8.41 and 8.38 (1H, dd, *J* = 1.2 and 3.6 Hz, H-4'), 8.28–8.15 (3H, m, H-2', H-9', H-7'), 7.97–7.87 (2H, m, H-3' and H-8'), 6.40 and 6.28

(1H, q,  $J = 6.3$  Hz, H-1''), 4.66 and 4.30 (1H, d,  $J = 7.8$  Hz, H-1 $\beta$ ), 4.06 (3H, s, OMe), 3.89 and 3.58 (1H, dd,  $J = 2.4$  and 12.0 Hz, H-6), 3.70 and 3.49 (1H, dd,  $J = 5.7$  and 12.0 Hz, H-6'), 3.45–3.21 (3H, m, H-2, H-3, H-4), 3.20–3.09 (1H, m, H-5), 1.70 and 1.68 (3H, d,  $J = 6.3$  Hz, H-2'').  $^{13}\text{C-NMR}$  for pairs of diastereomers (75 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  165.6, 142.3, 141.6, 133.4–127.8 (m, 10C), 101.9 and 101.2 (C-1 $\beta$ ), 76.9 and 76.8, 76.7 and 76.6, 74.3 and 74.2, 71.9, 70.5 and 70.4, 61.6 and 61.3 (C-1'), 52.0 (OMe), 23.1 and 21.7 (C-2'). Electrospray HR-MS (positive mode):  $m/z$  calcd. for  $\text{C}_{22}\text{H}_{25}\text{N}_2\text{O}_8$  (M + H) $^+$  445.1611, found 445.1598 (100%).

**Methyl 6-[(*R/S*)-1-( $\beta$ -D-galactopyranosyl)ethyl]phenazine-1-carboxylic ester (13).** 97% yield, pure  $\beta$ , 1 : 1 mixture of diastereomers ( $\beta$ -glycosides of both enantiomers of saphenic acid), mp 210–211 °C. Anal. HPLC (215 nm) >95%.  $^1\text{H-NMR}$  for pairs of diastereomers (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.42 and 8.40 (1H, dd,  $J = 1.5$  and 4.2 Hz, H-4'), 8.30–8.17 (3H, m, H-2', H-9', H-7'), 7.98–7.90 (2H, m, H-3' and H-8'), 6.41 and 6.28 (1H, q,  $J = 6.9$  Hz, H-1''), 4.61 and 4.25 (1H, d,  $J = 7.8$  Hz, H-1 $\beta$ ), 4.07 (3H, s, OMe), 3.82–3.32 (6H, m, H-2, H-3, H-4, H-5, H-6, H-6'), 1.70 and 1.68 (3H, d,  $J = 6.3$  Hz, H-2'').  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  167.6, 143.4, 141.8, 133.5, 133.4, 132.0, 131.5, 131.4, 129.3, 129.2, 128.5, 128.2, 126.2, 101.8 (C-1 $\beta$ ), 75.4 and 75.3, 73.9 and 73.8, 72.0 and 71.7, 71.6 and 70.3, 69.1 and 69.0, 61.3 and 60.9 (C-1'), 52.0 (OMe), 23.2 and 21.8 (C-2'). Electrospray HR-MS (positive mode):  $m/z$  calcd. for  $\text{C}_{22}\text{H}_{25}\text{N}_2\text{O}_8$  (M + H) $^+$  445.1611, found 445.1613 (100%).

**Methyl 6-[(*R/S*)-1-(6-deoxy- $\beta$ -L-glucopyranosyl)ethyl]phenazine-1-carboxylic ester (14).** 85% yield, pure  $\beta$ , 1 : 1 mixture of diastereomers ( $\beta$ -glycosides of both enantiomers of saphenic acid), mp 182–183 °C. Anal. HPLC (215 nm) >95%.  $^1\text{H-NMR}$  for pairs of diastereomers (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.30 and 8.26 (1H, d,  $J = 7.2$  Hz, H-4'), 8.16–8.10 (2H, m, H-9', H-7'), 7.97–7.91 (1H, d,  $J = 7.8$  Hz, H-2'), 7.77–7.69 (2H, m, H-3', H-8'), 6.13 (1H, m, H-1''), 4.57 and 4.37 (1H, d,  $J = 9.8$  Hz, H-1 $\beta$ ), 4.01 (3H, s, OMe), 3.50–3.08 (4H, m, H-2, H-3, H-4, H-5), 1.67 and 1.60 (3H, d,  $J = 7.2$  Hz, H-2''), 1.25 (3H, d,  $J = 2.4$  Hz, H-6).  $^{13}\text{C-NMR}$  for pairs of diastereomers (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.2, 143.9, 142.0, 141.9, 140.9, 140.8, 139.0, 133.7, 131.4, 130.2, 130.1, 129.3, 128.3, 89.2 (C-1 $\beta$ ), 75.5, 75.4, 72.2, 72.1, 60.6 (C-1'), 52.9 (OMe), 22.8 (C-2'), 17.9 (C-6). Electrospray HR-MS (positive mode):  $m/z$  calcd. for  $\text{C}_{22}\text{H}_{25}\text{N}_2\text{O}_7$  (M + H) $^+$  429.1662, found 429.1670 (100%).

**2-( $\beta$ -D-Glucopyranosyl)phenazine (15).** 95% yield, mp 236–237 °C (decomp.). Anal. HPLC (215 nm) >95%.  $^1\text{H-NMR}$  (500 MHz,  $\text{CD}_3\text{COOD}$ )  $\delta$  8.33–8.26 (3H, 3d,  $J = 7$ –8 Hz, H-4', H-6', H-9'), 7.95 (1H, br s, H-1'), 7.91 (1H, br t,  $J = 6.8$  Hz, H-7'/8'), 7.86 (1H, br t,  $J = 6.8$  Hz, H-7'/8'), 7.69 (1H, br d,  $J = 7.8$  Hz, H-3'), 5.41 (1H, d,  $J = 7.5$  Hz, H-1 $\beta$ ), 4.10 (1H, br d,  $J = 12.4$  Hz, H-6), 3.98 (1H, dd,  $J = 3.0$  and 12.4 Hz, H-6'), 3.93–3.77 (4H, m, H-2, H-3, H-4, H-5).  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CD}_3\text{COOD}$ )  $\delta$  160.3, 144.8, 143.4, 142.5, 141.4, 130.9, 130.8, 129.5, 129.4, 128.5, 128.2, 109.0 (C-1'), 100.9 (C-1 $\beta$ ), 86.4, 77.2, 77.1, 74.4, 70.6. Electrospray HR-MS (positive mode):  $m/z$  calcd. for  $\text{C}_{18}\text{H}_{19}\text{N}_2\text{O}_6$  (M + H) $^+$  359.1243, found 359.1232 (100%).

**2-( $\beta$ -D-Galactopyranosyl)phenazine (16).** 74% yield, mp 230–231 °C (decomp.). Anal. HPLC (215 nm) >95%.  $^1\text{H-NMR}$  (500 MHz,  $\text{CD}_3\text{COOD}$ )  $\delta$  8.36–8.29 (3H, 3d,  $J = 7$ –8 Hz, H-4', H-6', H-9'), 8.01 (1H, br s, H-1'), 7.93 (1H, br t,  $J = 7.9$  Hz, H-7'/8'), 7.89 (1H, br t,  $J = 7.5$  Hz, H-7'/8'), 7.73 (1H, br d,  $J = 8.0$  Hz, H-3'), 5.37 (1H, d,  $J = 7.5$  Hz, H-1 $\beta$ ), 4.23–4.08 (3H, m, H-6, H-6'), 4.03–3.95 (2H, m, H-3, H-4), 3.79 (1H, m, H-5).  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CD}_3\text{COOD}$ )  $\delta$  159.6, 145.1, 144.3, 143.9, 143.0, 141.0, 140.8, 136.7, 133.8, 133.5, 131.8, 108.9 (C-1'), 100.0 (C-1 $\beta$ ), 83.9, 80.9, 77.9, 71.4, 63.4. Electrospray HR-MS

(positive mode):  $m/z$  calcd. for  $\text{C}_{18}\text{H}_{19}\text{N}_2\text{O}_6$  (M + H) $^+$  359.1243, found 359.1266 (100%).

**2-( $\beta$ -L-6-Deoxygalactopyranosyl)phenazine (17).** 68% yield, mp 219–220 °C (decomp.). Anal. HPLC (215 nm) >95%.  $^1\text{H-NMR}$  (300 MHz,  $\text{CD}_3\text{COOD}$ )  $\delta$  8.38–8.30 (3H, m, H-4', H-6', H-9'), 7.98–7.85 (3H, m, H-1', H-7', H-8'), 7.71 (1H, dd,  $J = 2.7$  and 9.6 Hz, H-3'), 5.38 (1H, d,  $J = 7.5$  Hz, H-1 $\beta$ ), 3.94–3.85 (3H, m, H-2, H-3, H-4), 3.46 (1H, m, H-5), 1.46 (3H, d,  $J = 6.3$  Hz, H-6).  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CD}_3\text{COOD}$ )  $\delta$  159.6, 143.8, 142.3, 141.6, 140.5, 131.8, 130.4, 129.9, 128.7, 127.6, 126.9, 107.8 (C-1'), 100.0 (C-1 $\beta$ ), 76.4, 75.3, 73.8, 72.3, 17.1. Electrospray HR-MS (positive mode):  $m/z$  calcd. for  $\text{C}_{18}\text{H}_{19}\text{N}_2\text{O}_5$  (M + H) $^+$  343.1294, found 343.1205 (100%).

#### General procedure for the methyl ester hydrolysis

The methyl ester (**12**, **13** or **14**) and  $\text{LiOH}\cdot\text{H}_2\text{O}$  (5 equiv.) were dissolved in  $\text{MeOH-H}_2\text{O}$  (3 : 1) and stirred at room temperature for 19 h. The reaction mixture was acidified to pH 4 with  $\text{HCl}$  (aq. 4 M), evaporated to dryness, and purified by column chromatography on a Biotage QUAD3+ (eluting with  $\text{CH}_2\text{Cl}_2$ – $\text{MeOH}$  85 : 15 or 90 : 10) to give the free acids **18–20**.

**6-[(*R/S*)-1-( $\beta$ -D-Glucopyranosyl)ethyl]phenazine-1-carboxylic acid (18).** Quant. yield, 1 : 1 mixture of diastereomers ( $\beta$ -glycosides of both enantiomers of saphenic acid), oil. Anal. HPLC (215 nm) >95%.  $^1\text{H-NMR}$  (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.66 (1H, br d,  $J = 6.9$  Hz, H-2'), 8.45 (1H, br d,  $J = 8.7$  Hz, H-4'), 8.34 and 8.23 (1H, d,  $J = 6.9$  Hz, H-9'), 8.14–7.97 (3H, m, H-3', H-7', H-8'), 6.38 and 6.24 (1H, q,  $J = 6.3$  Hz, H-1''), 4.70 and 4.35 (1H, d,  $J = 7.2$  Hz, H-1 $\beta$ ), 3.90 and 3.61 (1H, dd,  $J = 2.4$  and 12.0 Hz, H-6), 3.73 and 3.50 (1H, dd,  $J = 5.4$  and 12.0 Hz, H-6'), 3.49–3.27 (3H, m, H-2, H-3, H-4), 3.20 (1H, m, H-5), 1.70 and 1.69 (3H, d,  $J = 6.3$  Hz, H-2'').  $^{13}\text{C-NMR}$  for pairs of diastereomers (75 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  174.7, 143.6, 142.7, 140.6, 140.1, 136.1, 134.9, 133.3, 130.1, 128.7, 128.4, 126.9, 126.6, 101.9 and 101.2 (C-1 $\beta$ ), 76.9 and 76.8, 76.7 and 76.6, 74.3 and 74.2, 71.8, 70.5 and 70.4, 61.6 and 61.4 (C-1'), 23.2 and 21.8 (C-2'). Electrospray HR-MS (positive mode):  $m/z$  calcd. for  $\text{C}_{21}\text{H}_{23}\text{N}_2\text{O}_8$  (M + H) $^+$  431.1454, found 431.1453 (100%).

**6-[(*R/S*)-1-( $\beta$ -D-Galactopyranosyl)ethyl]phenazine-1-carboxylic acid (19).** 97% yield, 1 : 1 mixture of diastereomers ( $\beta$ -glycosides of both enantiomers of saphenic acid), oil. Anal. HPLC (215 nm) >95%.  $^1\text{H-NMR}$  for pairs of diastereomers (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.55–8.18 (4H, m, H-2', H-4', H-9', H-3'), 8.10–7.91 (2H, m, H-7', H-8'), 6.41 and 6.28 (1H, q,  $J = 6.1$  Hz, H-1''), 4.62 and 4.65 (1H, d,  $J = 7.2$  Hz, H-1 $\beta$ ), 3.90–3.18 (6H, m, H-6, H-6', H-2, H-3, H-4, H-5), 1.71 and 1.69 (3H, d,  $J = 6.3$  Hz, H-2'').  $^{13}\text{C-NMR}$  for pairs of diastereomers (75 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  172.1, 143.3, 142.1, 141.7, 140.7, 138.5, 137.8, 134.8, 131.1, 130.1, 128.1, 127.9, 127.1, 102.8 and 101.8 (C-1 $\beta$ ), 75.4 and 75.3, 73.9 and 73.8, 71.7 and 71.6, 70.6, 69.1 and 68.9, 61.2 and 60.8 (C-1'), 23.2 and 21.8 (C-2'). Electrospray HR-MS (positive mode):  $m/z$  calcd. for  $\text{C}_{21}\text{H}_{23}\text{N}_2\text{O}_8$  (M + H) $^+$  431.1454, found 431.1443 (100%).

**6-[(*R/S*)-1-(6-Deoxy- $\beta$ -D-glucopyranosyl)ethyl]phenazine-1-carboxylic acid (20).** Quant. yield, 1 : 1 mixture of diastereomers ( $\beta$ -glycosides of both enantiomers of saphenic acid), mp 131–133 °C. Anal. HPLC (215 nm) >95%.  $^1\text{H-NMR}$  for pairs of diastereomers (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.80 (1H, br d, H-2'), 8.56 and 8.52 (1H, br d, H-4'), 8.34 and 8.20 (1H, d,  $J = 7.2$  Hz, H-9'), 8.22–8.00 (3H, m, H-3', H-7', H-8'), 6.31 and 6.21 (1H, q,  $J = 6.3$  Hz, H-1'), 4.62 and 4.28 (1H, d,  $J = 7.2$  Hz, H-1 $\beta$ ), 3.40–2.28 (4H, m, H-2, H-3, H-4, H-5), 1.70 and 1.68 (3H, d,  $J = 6$  Hz, H-2''), 1.29 and 0.92 (3H, d,  $J = 6$  Hz, H-6).  $^{13}\text{C-NMR}$  for pairs of diastereomers (75 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  167.0, 143.9,

142.8, 140.2, 140.0, 139.7, 135.4, 133.4, 133.3, 130.1, 128.6, 128.4, 126.5, 102.2 and 101.1 (C-1 $\beta$ ), 76.7 and 76.6, 75.9 and 75.8, 74.5 and 74.4, 72.1 and 71.9, 72.0 and 70.4, 23.2 and 21.8 (C-2'), 16.9 and 16.7 (C-6). Electrospray HR-MS (positive mode): *m/z* calcd. for C<sub>21</sub>H<sub>23</sub>N<sub>2</sub>O<sub>7</sub> (M + H)<sup>+</sup> 415.1505, found 415.1463 (100%).

## Acknowledgements

This work was financed in part by The Technical University of Denmark (JBL), The LEO Foundation (KJJ), The Benzon Foundation (KJJ), The Villum Kann Rasmussen Foundation (JN), and The Torkil Holm Foundation (JN). We thank Professor Klaus Bock, Carlsberg Research Center, for providing us with benzooylated quinovose. We thank Dr Hanne Theilgaard and Dr Anne Henriksen of LEO Pharma A/S for performing bacterial screening, and Dr Elisabeth V. Carstensen and her co-workers at TopoTarget A/S for performing topoisomerase II inhibition assays.

## References

- 1 D. J. Faulkner, *Nat. Prod. Rep.*, 1991, **8**(2), 97–147.
- 2 (a) K. H. Michel, M. M. Hoehn, *US Pat.*, 4316959, 1982 (*Chem. Abstr.* 1982, **96**, 197888); (b) W. Keller-Schierlein, A. Geiger, H. Zähler and M. Brandl, *Helv. Chim. Acta*, 1988, **71**, 2058–2070; (c) A. Geiger, W. Keller-Schierlein, M. Brandl and H. Zähler, *J. Antibiot.*, 1988, **41**, 1542–1551; (d) M. Kitahara, H. Nakamura, Y. Matsuda, M. Hamada, K. Meada, H. Umezawa and Y. Iitaka, *J. Antibiot.*, 1982, **35**, 1412–1414.
- 3 B.-S. Yun, I.-J. Ryoo, W.-G. Kim, J.-P. Kim, H. Koshino, H. Seto and I.-D. Yoo, *Tetrahedron Lett.*, 1996, **37**, 8529–8530.
- 4 L. Petersen, K. J. Jensen and J. Nielsen, *Synthesis*, 1999, **10**, 1763–1766.
- 5 J. B. Laursen, C. G. Jørgensen and J. Nielsen, *Bioorg. Med. Chem.*, 2003, **11**(5), 723–731.
- 6 J. B. Laursen, P. de Visser, H. K. Nielsen, K. J. Jensen and J. Nielsen, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 171–175.
- 7 (a) U. Hollstein and R. J. Van Gemert, Jr., *Biochemistry*, 1971, **10**, 497–504; (b) C. W. Mosher, D.-Y. Lee, R. M. Enanoza, P. A. Sturm and K. F. Kuhlmann, *J. Med. Chem.*, 1979, **22**, 918–922.
- 8 J. R. Kerr, *Infect. Dis. Rev.*, 2000, **2**(4), 184–194.
- 9 (a) S. A. Gamage, J. A. Spicer, G. J. Finlay, A. J. Stewart, P. Charlton, B. C. Baguley and W. A. Denny, *J. Med. Chem.*, 2001, **44**, 1407–1415; (b) J. A. Spicer, S. A. Gamage, G. J. Finlay and W. A. Denny, *Bioorg. Med. Chem.*, 2002, **10**, 19–29.
- 10 (a) M. Ge, Z. Chen, H. R. Onishi, J. Kohler, L. L. Silver, R. Kerns, S. Fukuzawa, C. Thompson and D. Kahne, *Science*, 1999, **284**, 507–511; (b) K. C. Nicolaou, H. J. Mitchell, F. L. van Delft, F. Rübsam and R. M. Rodriguez, *Angew. Chem., Int. Ed.*, 1998, **37**, 1871–1874.
- 11 (a) F. Arcamone, *Doxorubicin: Anticancer Antibiotics*, Academic Press, New York, 1981; (b) S. Penco and F. Arcamone, *Molecular aspects of anticancer drug action*, ed. J. W. Lown, Macmillan Press, London, 1988; (c) R. D. Anderson, M. L. Veigl, J. Baxter and W. D. Sedwick, *Cancer Res.*, 1991, **51**, 3930–3937.
- 12 D. B. Berkowitz, S. Choi, D. Bhuniya and R. K. Shoemaker, *Org. Lett.*, 2000, **2**(8), 1149–1152.
- 13 S. Erbeck, X. Liang, D. Hunkler, R. Krieger and H. Prinzbach, *Eur. J. Org. Chem.*, 1998, 1925–1948.
- 14 C. Pathirana, P. R. Jensen, R. Dwight and W. Fenical, *J. Org. Chem.*, 1992, **57**, 740–742.
- 15 (a) L. Petersen and K. J. Jensen, *J. Org. Chem.*, 2001, **66**, 6268–6275; (b) L. Petersen and K. J. Jensen, *J. Chem. Soc., Perkin Trans. 1*, 2001, 2175–2182; (c) J. B. Laursen, L. Petersen and K. J. Jensen, *Org. Lett.*, 2001, **3**, 687–690; (d) L. Petersen, J. B. Laursen, K. Larsen, M. S. Motawia and K. J. Jensen, *Org. Lett.*, 2003, **5**(8), 1309–1312.
- 16 For a review of glycosylation protocols under neutral or mild basic conditions, see: K. J. Jensen, *J. Chem. Soc., Perkin Trans. 1*, 2002, **20**, 2219–2233.
- 17 For a review of combinatorial synthesis of natural products, see: J. Nielsen, *Curr. Opin. Chem. Biol.*, 2002, **6**(3), 297–305.
- 18 Subjection of saphenic acid **4** to hydrogenolysis conditions (1 atm. H<sub>2</sub>, cat. Pd/C, MeOH–Hexane–CHCl<sub>3</sub> or AcOH, rt, 24 h) leads to complete decomposition of the phenazine structure.
- 19 (a) K. Ley and F. Seng, *Synthesis*, 1975, 415–422; (b) D. L. Vivian, *J. Am. Chem. Soc.*, 1951, **73**, 457–458.
- 20 B. Capon and S. P. McManus, *Neighbouring Group Participation*, Plenum Press, New York, 1976.
- 21 The compounds were screened in growth inhibition studies by LEO Pharma A/S on a panel of Gram-positive skin flora (*Micrococcus luteus*, *Propionibacterium acnes*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*) and on two resistant *Staphylococcus aureus* strains: clinical isolates of fusidic acid resistant and rifampicin resistant MRSA.
- 22 (a) S. W. Langer, G. Schmidt, M. Sørensen, M. Sehested and P. B. Jensen, *Clin. Cancer Res.*, 1999, **5**, 2899–2907; (b) B. B. Hasinoff, T. I. Kuschak, J. C. Yalowich and A. M. Creighton, *Biochem. Pharmacol.*, 1995, **50**(7), 953–958.