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A galabiose-based two-dimensional scaffold for the synthesis of inhibitors targeting P^k- and P-antigen binding proteins

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Abstract—A disaccharide scaffold based on galabiose (Galα1–4Gal) was synthesized. Four different acceptors were evaluated in the α-galactosylation and a relationship between the nucleophilicity, yield, and α/β-selectivity was found. The scaffold contains two orthogonal derivatisation sites, i.e. at O-2′ and the anomeric position, and as proof of concept, one derivatised galabioside was synthesized. Compounds based on this galabiose-scaffold are potential inhibitors of P- and P^k-antigen binding proteins.

The surface of an animal cell is covered with glycoproteins and glycolipids[1,2] and it is well known that pathogenic bacteria, viruses and toxins attach to these glycoconjugates.[3,4] This attachment is often a prerequisite for infection and/or toxicity and thus interesting as a target for the development of novel ways to treat bacterial and viral infections. Well-known examples, where carbohydrate-structures are exploited as attachment mediators by pathogens, are the P^k and P-antigen (Galα1–4Galβ1–4Glc and GalNAcβ1–3Galα1–4Galβ1–4Glc, respectively). These carbohydrate structures are in various stages of infection bound by uropathogenic *Escherichia coli*,[5,6] *Streptococcus suis*,[7,8] parvovirus B19,[9] Shiga toxin from *Shigella dysenteria*[10] and Verotoxins from *E. coli*.[11] A majority of these binding events have been demonstrated to require the galabiose (Galα1–4Gal) disaccharide present as a core structure in both the P- and P^k-antigen.

In the search for inhibitors of carbohydrate–lectin interactions, the introduction of substituents on core carbohydrate-structures as scaffolds has been successfully employed.[12–15] Within this context, a properly designed galabiose derivative can be used as a scaffold in the assembly of a combinatorial collection of compounds, aimed at finding inhibitors of P and P^k-binding proteins. We recently reported the use of a galabiose scaffold functionalised with a primary alkyl bromide at C-1 and an allyl ether at O-3′ in the combinatorial assembly of 20 galabiose-based inhibitors of PapG adhesins of uropathogenic *E. coli*.[15] Herein we describe the synthesis of a novel galabiose-based scaffold **1** (Fig. 1) that allows orthogonal introduction of substituents at O-2′ and C-1.

A galactose donor with a non-participating protecting group at O-2 was required in the synthesis of the scaffold **1**. To meet this requirement, a *p*-methoxybenzyl ether was chosen as protection group at O-2, because it possesses the additional feature of being removable under selective mildly acidic conditions.[16] Furthermore, a galactose acceptor with HO-4 unprotected and an azide at the anomeric position was required. α-Galactosylations of galactopyranose HO-4 are normally favoured by having a per-benzylated donor and a 2,3,6-tri-*O*-benzoyl-protected acceptor.[17] However, changing the 2-*O*-benzyl of the donor for a 2-*O*-*p*-methoxybenzyl (i.e. **10**[18]) and having an azide at the anomeric position of the acceptor (i.e. acceptor **6**) resulted in a low yield of the desired disaccharide, although with retained α-selectivity. In order to investigate this α-galactosylation further, a series of acceptors (**6**–**9**) with an increasing number of benzyl-groups was synthesized (Scheme 1) and evaluated. The synthesis of acceptors **6**–**9** (Scheme 1) started from galactosyl azide **2**[19] by introduction of a 4,6-*O*-benzylidene acetal

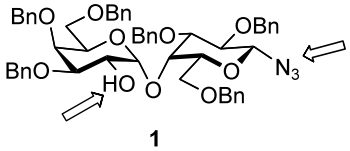

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Figure 1. Scaffold **1**.

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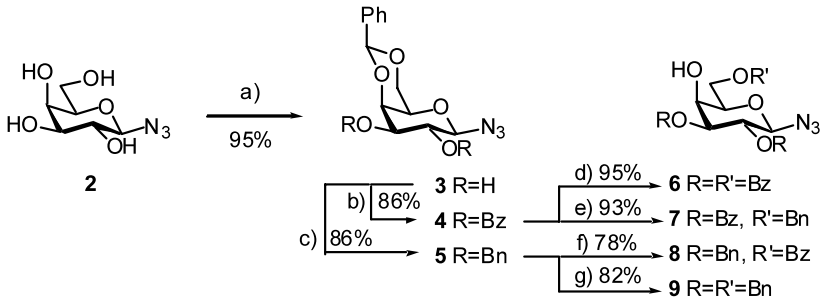


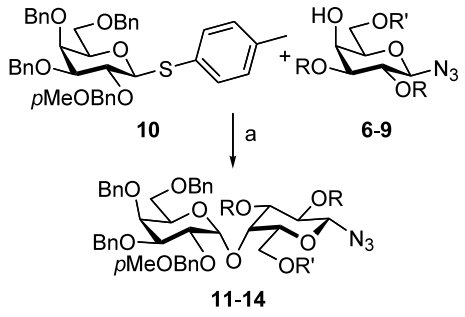
Scheme 1. *Reagents and conditions*: (a) α,α-dimethoxytoluene, *p*-TSA, CH_3CN. (b) BzCl, pyridine. (c) BnBr, NaH, DMF. (d) i. 80% aqueous AcOH, 80°C, ii. BzCl, pyridine, −10°C, 0.5 h. (e) $NaCNBH_3$, THF, MS AW-300, HCl–Et_2O (pH 2), 0°C, 4 h. (f) i. 80% aqueous AcOH, 80°C, ii. BzCl, pyridine, −10°C, 0.5 h. (g) $NaCNBH_3$, THF, MS AW-300, HCl–Et_2O (pH 2), 0°C, 30 min.

through treatment of **2** with α,α-dimethoxytoluene and a catalytic amount of *p*-toluenesulfonic acid in freshly distilled acetonitrile, which gave **3** in 95% yield. Compound **3** was either benzoylated by treatment with benzoyl chloride in pyridine to give galactoside **4** in 86% yield, or benzylated by treatment with benzyl bromide and sodium hydride in DMF to give galactoside **5** in 86% yield. The azide **4** was converted to acceptor **6** by hydrolysis of the benzylidene acetal in aqueous acetic acid at 80°C, followed by selective 6-*O*-benzoylation with benzoyl chloride in pyridine at −10°C. The galactosyl acceptor **7** was prepared from **4** by reductive opening of the 4,6-*O*-benzylidene ring with sodium cyanoborohydride in ethereal hydrogen chloride solution[20] in 93% yield. Finally, compounds **8** and **9** were prepared from **5** as described for **6** and **7**, in 78% and 82% yields, respectively.

Two trends could be observed when the four galactosyl acceptors **6**–**9** were α-galactosylated with the thioglycoside **10** under *N*-iodosuccinimide/trimethylsilyl trifluoromethanesulfonate[21,22] promotion, to give the galabiosides **11**–**14** (Table 1). First, the more benzyl groups on the acceptor, thereby increasing the acceptor nucleophilicity, led to increased yields. The major side product was identified as the succimido glycoside corresponding to **10** and with the more nucleophilic acceptors, this side reaction was suppressed. However, a second trend was that increasing the number benzyl groups on the galactosyl acceptor was accompanied by lowered α/β-selectivity. The introduction of two or three benzyls (i.e. entry 3 and 4, Table 1) led to the formation of the β-anomer, which was unseparable from the α-anomer. This is a typical example of the need for fine-tuning reaction conditions, i.e. matching acceptor nucleophilicity with donor reactivity, in glycosylations that proceed via the in situ anomerisation mechanism.[23]

The galactosyl acceptor **7** was chosen for further reactions since it provided the highest yield without detrimental formation of the β-anomer. Debenzoylation of galabioside **12** in methanolic sodium methoxide, followed by benzylation gave the benzyl protected galabioside **15** in 87% yield (Scheme 2). The *p*-methoxybenzyl group of **15** was smoothly cleaved with 2% trifluoroacetic acid in dichloromethane,[16] affording the target scaffold **1** in 95% yield.

Table 1. NIS/TMSOTf-Promoted glycosylations of **6**–**9** with **10**[a]

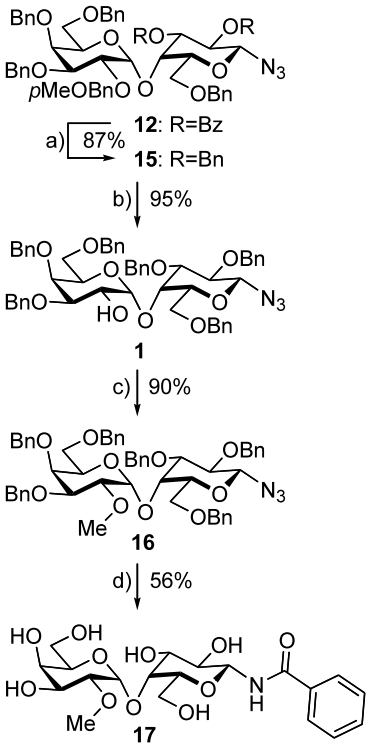


Entry	Acceptor	Product	Yield (%)	α/β
1	**6** R = R′ = Bz	11	49	>25/1
2	**7** R = Bz, R′ = Bn	12	67	>25/1
3	**8** R = Bn, R′ = Bz	13	76	12/1
4	**9** R = R′ = Bn	14	87	8/1

[a] Reaction conditions: *N*-Iodosuccinimide, TMSOTf, MS-AW300, CH_2Cl_2:Et_2O, 1:2, −50°C.

Compound **1** is ideal for the preparation of structurally diverse galabiose derivatives by alkylation of the unprotected HO-2′, followed by simultaneous removal of the benzyl groups and reduction of the azide and final *N*-acylation. In order to demonstrate the strategy (Scheme 2), the scaffold **1** was treated with methyl iodide and sodium hydride in DMF, yielding the intermediate galabioside **16** in 90% yield. The galabioside **16** was hydrogenolyzed over Pd/C in MeOH/HCl. *N*-Acylation was accomplished by the addition of Na_2CO_3 followed by dropwise addition of a solution of benzoyl chloride in THF. Purification on a Sep-Pak C18 cartridge afforded galabioside **17** along with about 15% methyl benzoate, which was easily removed with conventional flash chromatography yielding **17** in 56% yield.

In summary, we have reported an efficient synthesis of the galabiose scaffold **1**. Compound **1** can serve as a source for collections of diverse galabiose derivatives by orthogonal derivatization at HO-2′ and C-1. Such galabiosides are potential inhibitors of P^k- and P-antigen



Scheme 2. *Reagents and conditions*: (a) i. NaOMe, MeOH, CH_2Cl_2, ii. NaH, BnBr, DMF. (b) 2% TFA, CH_2Cl_2, 0°C. (c) NaH, MeI, DMF. (d) i. H_2, HCl (aq), Pd/C, MeOH, ii. BzCl, Na_2CO_3, THF.

binding proteins and constitute lead compounds towards the development of anti-adhesion therapeutical agents targeting these proteins. Finally, the attractive reactivity profile of the scaffold **1** (i.e. one hydroxyl ready for *O*-alkylation together with one azido group ready for subsequent reduction/acylation) may very well be generally useful within the field of combinatorial chemistry.

Acknowledgements

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