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Rewriting the bacterial glycocalyx *via* Suzuki–Miyaura cross-coupling†

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Suzuki–Miyaura cross-coupling has been used to couple novel carbohydrate-based boronic acids, site-selectively, to the surface of *E. coli* at an unnatural amino acid. In this way, benign metal-catalyzed cellular switching allowed modulation of interactions with biomolecular partners *via* prokaryotic *O*-glycosylation mimics.

Until recently protein glycosylation was thought to be a primarily eukaryotic phenomena.[1] However, since the discovery of the first prokaryotic glycoprotein in the archaebacteria *Halobacterium salinarium*,[2] and the first description of prokaryotic *N*-glycosylation in *Campylobacter jejuni*,[3] several cases have been described.[4] Indeed, whilst the full extent can only now be estimated, it is clear that prokaryotic protein glycosylation may be common, particularly on bacterial cell surfaces.[5]

Over the past decade much research has been undertaken to uncover the mechanisms and effects of glycosylation, and the selectivities towards interacting partners. Yet new tools are still required to allow further study and analysis.[6] Of particular appeal are new techniques for installing natural analogues or mimics of such glycosyl modifications in a site-specific manner, into relevant biomolecules and complex biological contexts.[7–9]

We have recently developed a water soluble, phosphine-free palladium catalyst ($Pd(OAc)_2(ADHP)_2$, **1**, see Fig. 1)[10] for protein modification *via* Suzuki–Miyaura cross-couplings[11] at genetically incorporated unnatural amino acids.[12] This system and related variants have subsequently shown utility in a variety of protein systems.[13,14] One such amino acid, the aryl halide *p*-iodophenylalanine (*p*IPhe), can be incorporated into both bacterial[15] and eukaryotic[16] systems site-selectively *via* amber-stop codon suppression.[17] We recently reported the use of this method to incorporate a *p*IPhe 'tag' onto cell surfaces and demonstrated the applicability of our catalyst for the cell surface Suzuki–Miyaura fluorescent labelling of 'tagged' cells.[18] In the course of this work, we demonstrated biological compatibility, as well as low associated toxicity. In addition, a critical Pd threshold effect was observed, leading to a 'switch-like' dose response.

Herein, we describe the synthesis of novel carbohydrate boronic acids and their cross-coupling to the surface of *E. coli*. This demonstrates the covalent conjugation of biomolecules to cell surfaces and the potential applicability of this system in the elucidation of prokaryotic glycobiology, through the selective modulation of cell surface binding partner interactions.

We envisaged the synthesis of both aryl **2** (as mimics of Tyr *O*-glycosylation)[19] and vinyl **10** (as mimics of Thr/Ser *O*-glycosylation)[20] boronic acids. D-Mannose (Man) and D-galactose (Gal) were chosen as suitable glycans as the most common hexose motifs found in natural glycoproteins,[21] whilst D-glucose (Glc) was also chosen due to its high natural abundance and widespread occurrence in biopolymers.[22]

To synthesise the D-galactoside aryl boronic acid, **2Gal**, the corresponding β-benzyl bromide, **3**, was first synthesized *via* BF_3-promoted glycosylation (Scheme 1a). Subsequent, Miyaura borylation[23] with bis(pinacolato)diboron gave the corresponding boronic pinacol ester, **4**. Removal of the ester was achieved through treatment with diethanolamine followed by acidic hydrolysis. Global Zemplén deprotection with NaOMe yielded free aryl sugar boronic acid **2Gal**.

The vinyl boronic acids were synthesised *via* two convergent methods (Scheme 1b). In our initial approach (Route 1), hydroboration of protected propargyl alcohol **5** and subsequent acidic deprotection gave the corresponding vinyl boronic ester alcohol, **6**, in good yields. Glycosylation of this acceptor, **6**, using the appropriate per-acetylated sugars as glycosyl donors was subsequently undertaken in the presence of a Lewis acidic promoter.[10] Whilst the use of BF_3·etherate proved sufficient for glycosylation using D-glucose and D-galactose donors in moderate yields (**9Glc** and **9Gal**), the use of TMSOTf was required to access the corresponding D-mannoside, **9Man**. Whilst complete consumption of the glycosyl donors was observed in all cases, overall reduced yield was attributed to hydrodeboronation of the product under the acidic reaction conditions, giving corresponding deborylated vinyl sugars as unwanted side products.[24]

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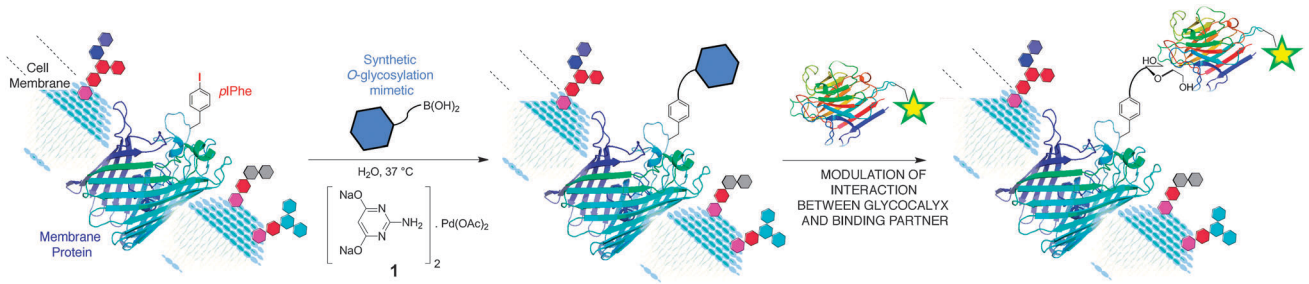
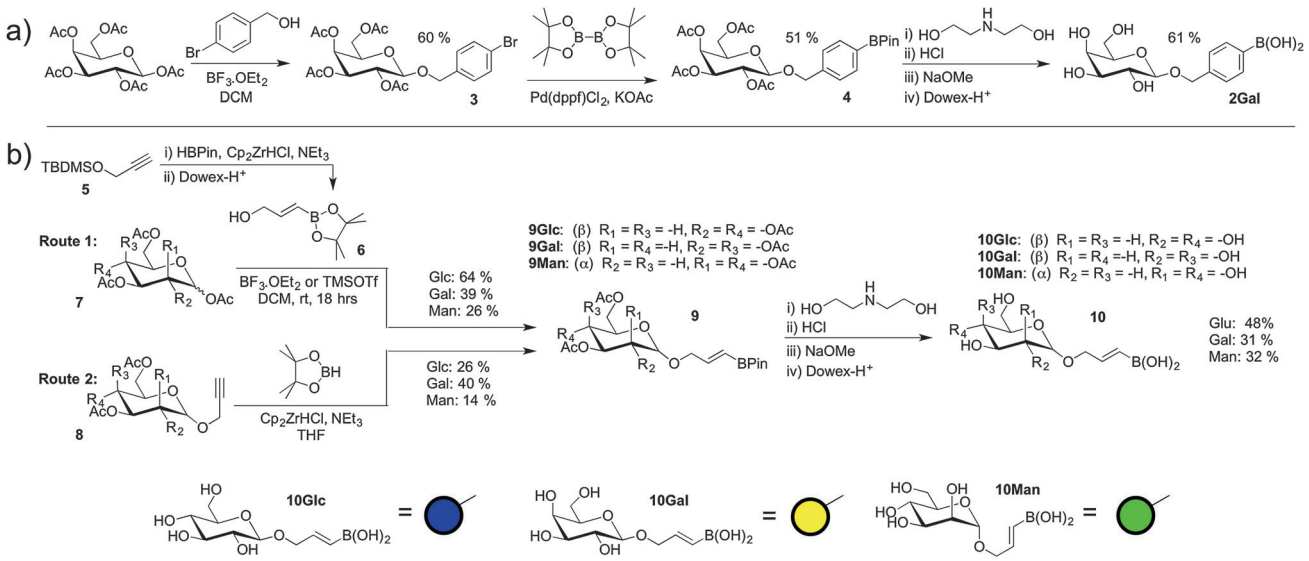


Fig. 1 Proposed procedure for cell surface carbohydrate modification of *E. coli*.



Scheme 1 Synthesis of sugar functionalised boronic acids **2Gal**, **10Glc**, **10Gal** and **10Man**.

As a result, we investigated alternative access (Route 2) to **9** *via* hydroboration of the corresponding propargyl derivatives, **8**, themselves synthesised *via* previously reported boron trifluoride promoted glycosylations.[25] A number of hydroboration conditions were investigated with little success: use of an excess of catecholborane[26] under solvent-less conditions failed to yield desired product, nor use of dibromoborane,[27] dicyclohexylborane[28] or diisopinocampheylborane.[29] However, the use of pinacolborane in the presence of catalytic amounts of Schwartz's reagent (Cp_2ZrHCl) and triethylamine[30] provided the protected boronic esters, **9**, albeit in modest yields. Deprotection of the boronic esters was again achieved with diethanolamine, followed by deprotection with sodium methoxide to yield the desired sugars, **10**, as crystalline solids (Scheme 1).

With these boronic acids (**2Gal**, **10Glc**, **10Gal** and **10Man**) in hand, Suzuki–Miyaura cross-couplings were undertaken on *p*IPhe 'tagged' *E. coli*.[18] Briefly, *E. coli* JW2203-1 (an OmpC knockout strain from the Keio collection[31]) was co-transformed with plasmids pEVOL(IPhe)[17] and pOmpC-(Y232·), to allow amber-suppressed incorporation of *p*IPhe into a mutant OmpC membrane protein at position Y232 (see ESI†). After overnight induction of protein expression with 1 mM IPTG, 0.02% L-arabinose and 2 mM *p*IPhe, cells were collected by centrifugation and washed extensively with pH 8.0 phosphate buffer. The cells were then labelled at an OD_{600} = 0.2 using a palladium concentration of 0.5 mM and a boronic acid concentration of 3.5 mM. After labelling at 37 °C for 1 h, cells were collected by centrifugation and washed extensively to ensure the complete removal of unreacted boronic acid. Disappointingly, the Gal-tyrosinyl mimic reagent **2Gal** proved to be poorly soluble in water under conditions amenable to biological systems and so could not be used for cellular labelling. The glycosyl-serinyl mimic reagents **10Glc**, **10Gal** and **10Man**, however, were all highly soluble under relevant conditions and proved to be highly effective coupling partners.

Having thus synthetically altered the composition of the cell surface glycocalyx through C–C coupling, we investigated the modulation of its interactions with glycan-selective binding protein partners. *Lens culinaris* agglutinin (LCA)[32] and *Griffonia simplicifolia* lectin I (GSL)[33] were chosen as α-Man- and β-Gal-selective lectins, respectively, whilst Concanavalin A (ConA) was used as a positive control due to its known affinity for the existing glycan lipo-polysaccharide (LPS) of *E. coli* outer membranes.[34] Gratifyingly, when cell surface glycans were 'switched' to alternatives *via* Suzuki–Miyaura cell-surface modification, a high specificity of recognition was observed. Modulation of the cell surface with Gal (from **10Gal**) resulted in strong interaction with GSL (Fig. 2 middle), whilst synthetic 'switching' to Man (from **10Man**) resulted in a high interaction

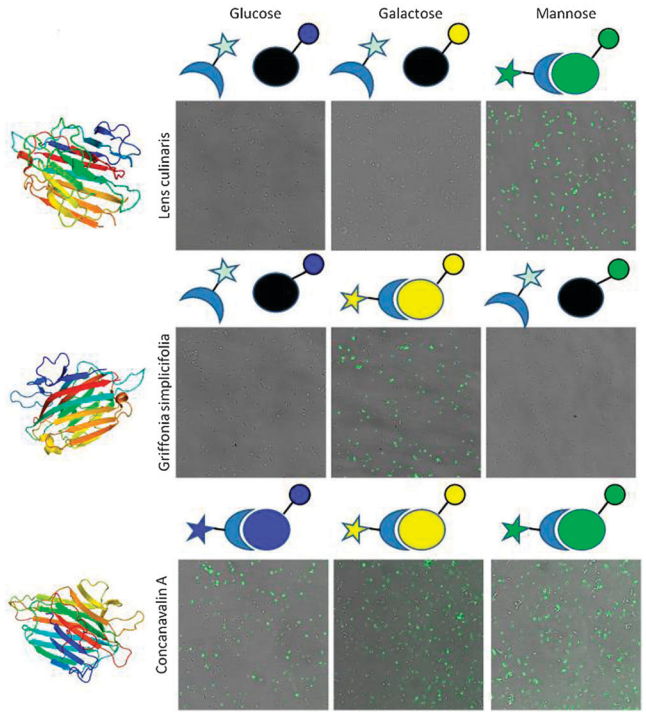


Fig. 2 Interaction of fluorescein–lectin conjugates with *E. coli* labelled with monosaccharide boronic acids *via* Suzuki–Miyaura coupling.

specificity for LCA (Fig. 2 top). Glc modification of the cell surfaces resulted in no interaction with either of these proteins. Experiments run in the absence of the key coupling partners (palladium, boronic acid or in cells grown in the absence of *p*IPhe) also failed to result in any binding, strongly supporting a Suzuki–Miyaura-induced switching mechanism (see ESI†). Notably, the existing, natural *E. coli* glycocalyx was not disrupted during the course of the reaction, allowing the inherent interaction with ConA to be maintained in all cases (Fig. 2 bottom). In addition to confirming Suzuki–Miyaura glycoconjugations at bacterial cell surfaces, these results also demonstrated the accessibility and potential applicability of these biologically relevant ligands for future applications.

In conclusion, we have synthesised a series of novel carbohydrate based boronic acids, and applied these as cross-coupling partners for the Suzuki–Miyaura labelling of *p*IPhe 'tagged' cells. The cognate interactions of these biologically relevant ligands could be visualised on the cell surface *via* the selective binding of fluorescein–lectin conjugates, thus demonstrating the functionality of such ligands in a complex and relevant biological context. Importantly, we have also demonstrated[18] low associated toxicity. The use of such couplings further demonstrates the power of Pd-mediated control of Biology,[35] here in cellular interactions. We are currently working towards utilising such methods in eukaryotic systems, particularly in the potential blood typing ('blood groups') of mammalian samples.

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