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# Fluorescence labelling of carbohydrates with 2-aminobenzamide (2AB)

Robert R. France,[a] Ian Cumpstey,[a] Terry D. Butters,[b] Antony J. Fairbanks[a,*] and Mark R. Wormald[b]

[a]*Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY, UK*
[b]*Oxford Glycobiology Institute, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK*

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**Abstract**

2-Aminobenzamide (2AB) is a common fluorescence label attached to reducing oligosaccharides by a reductive amination procedure. Chemical investigation of the published literature procedure reveals labelling occurs by the expected mechanism for both protected and unprotected glucose derivatives to yield open-chain carbohydrates rather than result in the formation of any heterocyclic materials. Pentenyl glucosides may also be readily attached to the 2AB label by a sequence of dihydroxylation, periodate cleavage and subsequent reductive amination of the resulting aldehyde. 2AB labelling is compatible with deprotection of both acetate and benzyl protecting groups. 

## 1. Introduction

Fluorescence labels are extremely useful tools since they allow the monitoring of extremely low concentrations of particular chemical species involved in a biochemical process of interest. Fluorescence labelling of carbohydrates is commonly used to visualise oligosaccharides[1] after cleavage from glycoproteins in order either to facilitate purification of the oligosaccharide of interest,[2] or to allow monitoring of the concentration of a particular oligo- or monosaccharide as the substrate for a particular enzyme.[3]

We recently became interested in the synthesis of a series of substrates for the glycoprotein processing enzymes glucosidase I and II and the question arose as to the best method of attaching fluorescence labels to synthetic substrates for these enzymes. Anthranilamide (2-aminobenzamide, 2AB) is a frequently used fluorescence label for the visualisation of oligosaccharide materials.[4] We envisaged that attachment of a 2AB label to a carbohydrate by means of

* Corresponding author. Tel: +44-1865-275647; fax: +44-1865-275674; e-mail: antony.fairbanks@chem.ox.ac.uk

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an *n*-pentenyl glycoside[5] derived spacer arm would allow a flexible approach since upon activation *n*-pentenyl glycosides can act as efficient glycosyl donors. Thus other sugars could be added before labelling if required by taking advantage of an armed/disarmed approach.[6] We detail herein our investigations into the attachment of a 2-aminobenzamide label to some glucose derivatives by reductive amination procedures, either directly at the anomeric centre or via a pentenyl derived spacer arm.

## 2. Results and discussion

At the outset of our investigations we became aware of the fact that no thorough chemical investigation concerning the efficiency or mode of linking of the 2AB label to the anomeric centre of the reducing terminus of an oligosaccharide had been published.[7] In fact to our surprise we were unable to find any reports of reductive amination of aldehydes with 2AB in the chemical literature. This is doubly surprising considering that 2AB labelling is now considered a standard practice in many glycobiology laboratories, frequently employing a commercially available reaction kit. It is well known that tetrahydroquinazolinones can result from reaction of anthranilamide with aldehydes[8] (Fig. 1), or with ketones under more forcing conditions.[9] At the outset it was therefore not completely clear to us that reductive amination 'as expected' was necessarily the sole outcome of previously reported procedures and therefore complete characterisation of the 2AB labelled carbohydrates synthesised was prudent.

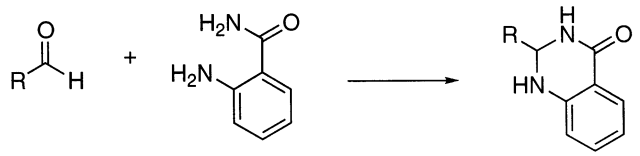
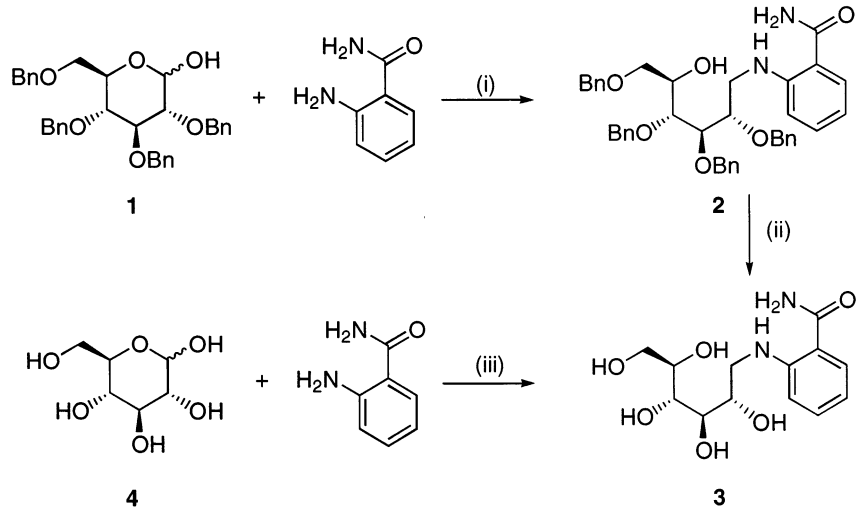



Figure 1.

Initial investigations centered on the 2AB labelling of tetrabenzylglucose **1**, following the published protocol of Bigge et al.[5] (Scheme 1). Treatment of **1** with sodium cyanoborohydride in a mixture of dimethyl sulphoxide and acetic acid at 60°C did result in production of the labelled open-chain sugar **2** as the major reaction product, in an acceptable 70% yield. The open-chain structure of **2** was confirmed by $^1$H NMR and mass spectral analysis; in particular the two H-1 protons could be clearly discerned. It should be noted that a mixture of several other unidentified minor side products, which were inseparable by flash chromatography, was also produced during this reaction. This mixture of side products could well contain tetrahydroquinazolinone materials, but proper characterisation of the complex mixture proved difficult. Removal of the benzyl protecting groups from **2** proved more problematic then expected. Simple catalytic hydrogenation with hydrogen gas and a selection of heterogeneous Pd catalysts produced no reaction. However refluxing **2** in methanol in the presence of excess palladium on carbon and ammonium formate for 24 hours finally successfully yielded the completely deprotected material **3** in 60% yield. 2-Aminobenzamide labelling of glucose **4** itself was then investigated using the same reaction conditions. Again labelling occurred as expected and the desired product **3** was obtained, though in a moderate 67% yield; it should be noted that the isolation and purification of this highly polar compound is not facile. It is therefore clear that whilst anthranilamide labelling using the published literature procedure does not quantita-

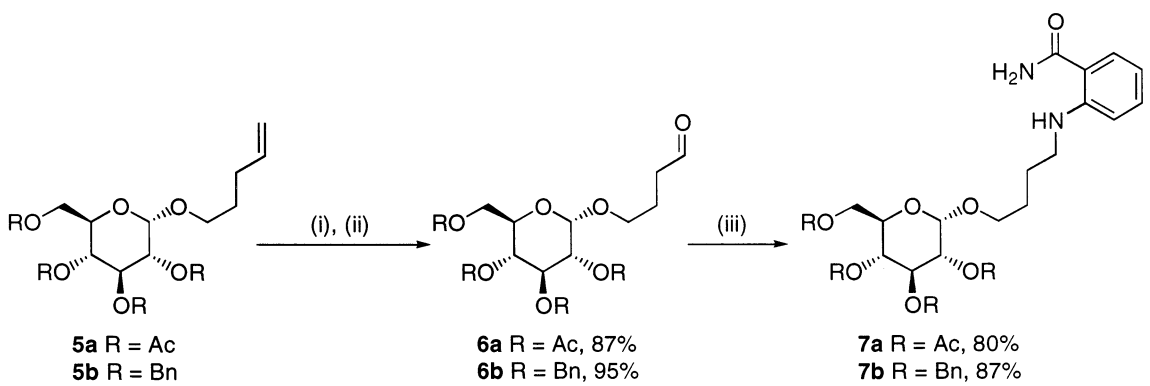



Scheme 1. (i) $NaBH_3CN$, $Me_2SO$, AcOH, 60°C, 24 h, 70%; (ii) ammonium formate, Pd/C, MeOH, reflux, 24 h, 60%; (iii) $NaBH_3CN$, $Me_2SO$, AcOH, 60°C, 2 h, 67%

tively result in the formation of a single reaction product, that the major reaction product is indeed the desired reductively aminated material, rather than a tetrahydroquinazolinone.

Attention then moved towards the use of *n*-pentenyl glycosides as potential sites for the attachment of 2AB labels. As previously discussed this approach would be inherently flexible, since a pentenyl linker arm could also be activated for the addition of other carbohydrate units if desired. The ultimate goal of this research was the synthesis of a series of fluorescence labelled oligosaccharides as substrates for a variety of glycosidases. To this end it was also thought that the pentenyl derived spacer arm would be appropriate mode of attachment for the 2AB label since the alkyl chain would hopefully be long enough to avoid any appreciable interaction between the label and the enzyme.

The peracetylated and perbenzylated α-gluco pentenyl glycosides **5a** and **5b** were synthesised from glucose following standard literature procedures.[10] Conversion of the alkene to an aldehyde ready for reductive amination was best achieved by the standard two step variation involving dihydroxylation and periodate cleavage, producing aldehydes **6a** and **6b** in 87 and 95% yields, respectively (Scheme 2). Attempted ozonolysis was not clean, and resulted in the formation of the carboxylic acid as an undesired side product in both cases.




**5a** R = Ac
**5b** R = Bn

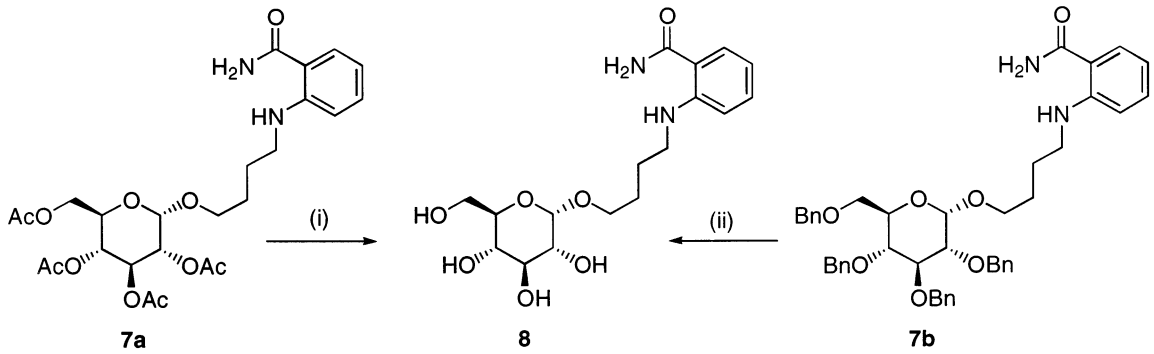
**6a** R = Ac, 87%
**6b** R = Bn, 95%

**7a** R = Ac, 80%
**7b** R = Bn, 87%

Scheme 2. (i) $OsO_4$, *N*-methylmorpholine *N*-oxide, THF; (ii) $NaIO_4$, THF, $H_2O$; (iii) $NaBH(OAc)_3$, DCE, rt, 2AB

A screen of conditions for the reductive amination reaction revealed that the best reaction conditions involved the use of sodium triacetoxyborohydride[11] in 1,2-dichloroethane (DCE), which resulted in the formation of the 2AB labelled α-glucosides **7a** and **7b** in 80 and 87% yields, respectively. It should be noted that in the case of the perbenzylated aldehyde **6b** the previously used reaction conditions employing sodium cyanoborohydride resulted in the formation of an intractable complex mixture of products.

Finally deprotection of both labelled materials was undertaken in an attempt to access the desired tetrol **8**. Deacetylation of **7a** occurred rapidly with catalytic methoxide in methanol to yield **8** in a satisfactory 63% yield (Scheme 3). However, removal of the benzyl protecting groups of **7b** proved problematic. Initial attempts at simple hydrogenation with hydrogen gas and catalytic Pd black resulted in no reaction. A screen of various catalysts for hydrogenation was subsequently undertaken. After many unsuccessful attempts it was discovered that once again complete removal of all benzyl groups could be achieved by prolonged reflux in methanol with a large excess of ammonium formate and palladium on carbon. Thus finally all benzyl groups were removed to yield **8** in a satisfactory 76% yield.




Scheme 3. (i) NaOMe, MeOH, rt, 4 h, 63%; (ii) ammonium formate, Pd/C, MeOH, reflux, 36 h, 76%

In summary in the case of reducing sugars it is clear that labelling at the anomeric centre of protected carbohydrates with the anomeric hydroxyl free using 2AB and sodium cyanoborohydride mediated reductive amination is reasonably efficient. It is also clear that fluorescence labels such as 2AB may be attached to pentenyl glycosides by a sequence involving dihydroxylation, periodate cleavage and reductive amination. In addition pentenyl linked 2AB labels are compatible with both ester and benzyl ether protecting groups. However the difficulties encountered during attempted hydrogenation steps should be borne in mind when choosing a protecting group pattern; in particular the current conditions require large amounts of catalyst and protracted reaction times. Further investigations into more efficient methods of benzyl group removal, and into the attachment of various different fluorescence labels to pentenyl glycosides are currently in progress and will be reported in due course.

## 3. Experimental

### 3.1. General methods

Petrol refers to the fraction of petroleum ether that boils in the range 40–60°C; water was distilled. All other solvents were used as supplied (analytical grade) without prior purification. Reactions performed under an atmosphere of hydrogen gas were maintained by an inflated balloon. All other reagents were used as supplied without prior purification. Thin layer chromatography (TLC) was performed on glass backed plates coated with 60 F254 silica. Plates were visualised using a solution of 5% ammonium molybdate in 2 M sulphuric acid, and by fluorescence where appropriate. Flash column chromatography was performed on Sorbsil C60 40/60 silica. CMAW refers to the eluent system chloroform:methanol:acetic acid:water (60:30:3:5). Melting points were recorded on a Kofler hot block and are uncorrected. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter with a path length of 1 dm. Concentrations are quoted in g/100 ml. Elemental analyses were performed by the microanalysis services of the Inorganic Chemistry Laboratory (Oxford) and Elemental Microanalysis Ltd (Devon). Infrared spectra were recorded on a Perkin–Elmer 1750 IR Fourier Transform spectrophotometer using either thin films on NaCl discs (thin film) or KBr discs (KBr) as stated. Only the characteristic peaks are quoted. Nuclear magnetic resonance (NMR) spectra were recorded on a a Bruker DPX 400 ($^1$H: 400 MHz and $^{13}$C: 100 MHz), or where stated on Bruker DRX 500 or AMX 500 ($^1$H: 500 MHz and $^{13}$C: 125 MHz), in the deuterated solvent stated. All chemical shifts ($\delta$) are quoted in ppm. Residual signals from the solvents were used as an internal reference. Low resolution mass spectra ($m/z$) were recorded on a Micromass Platform APCI using atmospheric pressure chemical ionisation (APCI), and partial purification by HPLC with methanol:acetonitrile:water (40:40:20) as eluent. HRMS (electrospray) analyses were performed on a Waters 2790 Micromass LCT electrospray ionisation mass spectrometer or by the EPSRC Mass Spectrometry Service Centre, Department of Chemistry, University of Wales, Swansea on a MAT900 XLT electrospray ionisation mass spectrometer.

### 3.2. 2-(N-(1-Deoxy-2,3,4,6-tetra-O-benzyl-D-glucitol-1-yl)amino)benzamide **2**

Tetrabenzylglucose **1** (500 mg, 0.92 mmol) and anthranilamide (190 mg, 1.39 mmol) were dissolved in DMSO (3.5 ml) and AcOH (1.5 ml). After 30 min sodium cyanoborohydride (260 mg, 4.33 mmol) was added and the reaction mixture was heated to 60°C. The mixture was stirred at 60°C for 24 h, at which point TLC (ethyl acetate:petrol, 3:2) showed the formation of a major product ($R_f$ 0.4) and the absence of starting material ($R_f$ 0.6). The reaction mixture was cooled and partitioned between diethyl ether (100 ml) and 1.0 M HCl (100 ml). The organic layer was washed with brine (50 ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate:petrol:triethylamine, 1:2:0.5) to afford **2** (430 mg, 70%) as a colourless oil; $[\alpha]_D^{21}$ +0.4 ($c$, 1.0 in $CHCl_3$); $\nu_{max}$ (thin film) 3344 (NH), 1654 (C=O), 1616, 1579 (C=O, NH) cm$^{-1}$; $\delta_H$ (400 MHz, $CDCl_3$) 3.21 (1H, br, s, OH), 3.27 (1H, dd, $J_{1,1'}$ 13.5 Hz, $J_{1,2}$ 6.7 Hz, H-1), 3.45 (1H, dd, $J_{1',2}$ 4.3 Hz, H-1′), 3.64 (2H, d, $J_{5,6}$ 4.5 Hz, 2×H-6), 3.79 (1H, dd, $J_{3,4}$ 4.1 Hz, $J_{4,5}$ 6.8 Hz, H-4), 3.90 (1H, dd, $J_{2,3}$ 5.4 Hz, H-3), 3.98–4.02 (1H, m, H-2), 4.03–4.07 (1H, m, H-5), 4.49, 4.53 (2H, ABq, $J_{AB}$ 11.9 Hz, $PhCH_2$), 4.57, 4.61 (2H, ABq, $J_{AB}$ 11.5 Hz, $PhCH_2$), 4.62, 4.71 (2H, ABq, $J_{AB}$ 11.3 Hz, $PhCH_2$), 4.69 (2H, s, $PhCH_2$), 5.77 (2H, br, s, $NH_2$), 6.57–6.61 (2H, m, 2×ArH), 7.19–7.36 (22H, m, 22×ArH); $\delta_C$

($CDCl_3$) 43.9 (t, C-1), 71.3 (d, C-5), 71.7 (t, C-6), 73.7, 73.9, 75.0 (3×t, 4×$PhCH_2$), 78.0 (d, C-2), 78.1 (d, C-4), 79.4 (d, C-3), 112.5, 115.1, 133.9 (3×d, 3×ArC), 114.0, 150.5 (2×s, 2×ArC), 128.2, 128.3, 128.4, 128.7, 128.9, 128.9, 129.0 (7×d, 21×ArC), 138.4, 138.5 (2×s, 5×ArC), 172.8 (s, $CONH_2$); *m*/*z* (APCI$^+$) 717 ($MK^+H_2O$, 14%); HRMS calcd for $C_{41}H_{45}O_6N_2$ ($MH^+$): 661.3277; found: 661.3280.

### 3.3. 2-(N-(1-Deoxy-D-glucitol-1-yl)amino)benzamide **3**

#### 3.3.1. Method 1

A mixture of compound **2** (100 mg, 0.15 mmol), palladium on carbon (322 mg, 3.0 mmol), and ammonium formate (334 mg, 5.3 mmol) were stirred together in methanol (5 ml) and the reaction mixture heated under reflux. After 24 h, TLC (CMAW) showed the formation of a major product ($R_f$ 0.5). The reaction mixture was cooled, filtered, and the residue purified by flash column chromatography (CMAW) to afford **3** (27 mg, 60%) as a crystalline white solid, mp 171–174°C (EtOH); $[\alpha]_D^{24}$ −0.3 (*c*, 0.5 in $H_2O$); $\nu_{max}$ (KBr) 3400, 3252 (br, OH, NH), 1650 (C=O); $\delta_H$ (500 MHz, $CD_3OD/D_2O$, 1:1) 3.24 (1H, dd, $J_{1,1'}$ 13.1 Hz, $J_{1,2}$ 8.0 Hz, H-1), 3.45 (1H, dd, $J_{1',2}$ 4.1 Hz, H-1′), 3.66 (1H, dd, $J_{5,6}$ 5.6 Hz, $J_{6,6'}$ 11.6 Hz, H-6), 3.68 (1H, dd, $J_{3,4}$ 2.1, $J_{4,5}$ 8.8 Hz, H-4), 3.76–3.79 (1H, m, H-5), 3.82 (1H, dd, $J_{5,6'}$ 3.1 Hz, H-6′), 3.87 (1H, dd, $J_{2,3}$ 5.4 Hz, H-3), 4.02 (1H, dat, H-2), 6.71 (1H, at, *J* 7.5 Hz, ArH), 6.86 (1H, d, *J* 8.1 Hz, ArH), 7.38–7.41 (1H, m, ArH), 7.56 (1H, dd, *J* 7.9 Hz, *J* 1.5 Hz, ArH); $\delta_C$ ($CD_3OD/D_2O$, 1:1) 45.6, 48.6 (2×t, C-1, C-6), 71.0, 71.6, 71.7, 72.1 (4×d, C-2, C-3, C-4, C-5), 112.6, 116.1, 129.3, 133.9 (4×d, 4×ArC), 149 (s, ArC), 174.3 (s, $CONH_2$); *m*/*z* (APCI$^+$) 323 ($MNa^+$, 9), 301 ($MH^+$, 63), 120 (100%); HRMS calcd for $C_{13}H_{21}O_6N_2$ ($MH^+$): 301.1400; found: 301.1399.

#### 3.3.2. Method 2

Glucose **4** (205 mg, 1.14 mmol) and anthranilamide (230 mg, 1.69 mmol) were dissolved in DMSO (3.5 ml) and AcOH (1.5 ml). The reaction mixture was heated to 60°C and sodium cyanoborohydride (320 mg, 5.01 mmol) was added. The mixture was stirred at 60°C for 6 h, at which point TLC (CMAW) showed the formation of a major product ($R_f$ 0.5) and the absence of starting material ($R_f$ 0.2). The reaction mixture was cooled and the solvents removed in vacuo. The residue was dissolved in 1.0 M HCl (20 ml) and stirred for 1 h. The solvent was then removed in vacuo and the residue purified by flash column chromatography (CMAW) to afford **3** (227 mg, 67%) as a colourless oil, identical to the material described above.

### 3.4. 4-Oxobutyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside **6a**

Peracetylated pentenyl glycoside **5a**[10] (1.5 g, 3.61 mmol) and *N*-methylmorpholine *N*-oxide (60% solution in water, 1.1 ml, 5.61 mmol) were dissolved in THF (6 ml). Osmium tetroxide (11 mg, 0.043 mmol) was added and the reaction mixture was stirred for 17 h, at which point TLC (ethyl acetate) indicated the formation of a major product ($R_f$ 0.2) together with some remaining starting material ($R_f$ 0.7). The reaction mixture was quenched by the addition of sodium thiosulphate (4 ml of a saturated aqueous solution), and then stirred for a further 7 h. The mixture was diluted with ethyl acetate (100 ml) and washed with water (100 ml). The combined aqueous extracts were re-extracted with ethyl acetate (100 ml), and the combined organic layers washed with brine (100 ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was dissolved in a mixture of THF (8 ml) and water (1 ml), and sodium periodate (1.9 g, 9.02 mmol)

was then added. After 16 h, TLC (ethyl acetate:petrol:triethylamine, 2:3:0.25) showed the formation of a major product ($R_f$ 0.3). The reaction mixture was diluted with ethyl acetate (100 ml), washed with water (100 ml) and brine (100 ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate:petrol:triethylamine, 2:3:0.25) to give aldehyde **6a** (1.3 g, 87%) as a colourless oil; $[\alpha]_D^{21}$ +102 (*c*, 1.1 in $CHCl_3$); $\nu_{max}$ (thin film) 1750 ($OCOCH_3$) $cm^{-1}$; $\delta_H$ (400 MHz, $CDCl_3$) 1.93–2.17 (2H, m, $OCH_2C\underline{H}_2CH_2$), 2.01, 2.03, 2.07, 2.09 (12H, 4×s, 4×$CH_3$), 2.51–2.58 (2H, m, $OCH_2CH_2C\underline{H}_2$), 3.41–3.49 (1H, m, $OC\underline{H}_aH_bCH_2CH_2$), 3.72–3.82 (1H, m, $OCH_a\underline{H}_bCH_2CH_2$), 4.00 (1H, ddd, $J_{4,5}$ 10.3 Hz, $J_{5,6}$ 4.2 Hz, $J_{5,6'}$ 2.3 Hz, H-5), 4.08 (1H, dd, $J_{6,6'}$ 12.4 Hz, H-6), 4.25 (1H, dd, H-6′), 4.85 (1H, dd, $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 10.3 Hz, H-2), 5.02–5.09 (1H, m, H-4), 5.05 (1H, d, H-1), 5.46 (1H, at, *J* 9.8 Hz, H-3), 9.80 (1H, t, *J* 1.4 Hz, $CH_2C\underline{H}O$); $\delta_C$ ($CDCl_3$) 20.5, 20.6, 20.7 (3×q, 4×$CH_3$), 24.4, 30.3, 60.4, 67.4 (4×t, $O\underline{C}H_2\underline{C}H_2\underline{C}H_2$, C-6), 67.4, 68.4, 70.1, 70.8 (4×d, C-2, C-3, C-4, C-5), 95.7 (d, C-1), 169.6, 170.3, 170.7, 177.5 (4×s, 4×$\underline{C}O_2CH_3$), 201.5 (s, $CH_2\underline{C}HO$); *m*/*z* ($APCI^+$) 457 ($MK^+$, 6), 441 ($MNa^+$, 12), 169 (100%); anal. found: C, 49.64; H, 6.14; $C_{18}H_{26}O_{11}\cdot H_2O$ requires: C, 49.54; H, 6.47%; HRMS calcd for $C_{18}H_{30}O_{11}N$ ($MNH_4^+$): 436.1819; found: 436.1817.

### 3.5. 4-Oxobutyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside **6b**

Perbenzylated pentenyl glycoside **5b**[10] (454 mg, 0.75 mmol) and *N*-methylmorpholine *N*-oxide (60% solution in water, 0.21 ml, 1.11 mmol) were dissolved in THF (15 ml). Osmium tetroxide (8.1 mg, 0.032 mmol) was added and the reaction mixture was stirred for 18 h, at which point TLC (ethyl acetate) indicated a major product ($R_f$ 0.4) together with some starting material ($R_f$ 0.75). The reaction mixture was quenched by the addition of sodium thiosulphate (8 ml of a saturated aqueous solution), and then stirred at room temperature for a further 6 h. The reaction mixture was diluted with ethyl acetate (100 ml) and washed with water (2×50 ml). The combined aqueous extracts were re-extracted with ethyl acetate (100 ml), and the combined organic layers were washed with brine (100 ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was dissolved in a mixture of THF (8 ml) and water (1 ml), and sodium periodate (317 mg, 1.48 mmol) was added. After 15 h, TLC (ethyl acetate) showed the formation of a major product ($R_f$ 0.75), and the absence of any diol ($R_f$ 0.4). The reaction mixture was diluted with ethyl acetate (200 ml), washed with water (2×100 ml) and brine (50 ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate:petrol, 1:3) to give aldehyde **6b** (427 g, 95%) as a colourless oil; $[\alpha]_D^{21}$ +36 (*c*, 1.0 in $CHCl_3$); $\nu_{max}$ (thin film) 1723 (C=O) $cm^{-1}$; $\delta_H$ (400 MHz, $CDCl_3$) 1.91–2.06 (2H, m, $OCH_2C\underline{H}_2CH_2$), 2.54–2.59 (2H, m, $OCH_2CH_2C\underline{H}_2$), 3.43 (1H, dt, $J_{gem}$ 9.9 Hz, *J* 6.2 Hz, $OC\underline{H}_aH_bCH_2CH_2$), 3.56 (1H, dd, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.7 Hz, H-2), 3.61–3.76 (5H, m, H-4, H-5, H-6, H-6′, $OCH_a\underline{H}_bCH_2CH_2$), 3.96 (1H, at, *J* 9.3 Hz, H-3), 4.47, 4.84 (2H, ABq, $J_{AB}$ 10.7 Hz, $PhCH_2$), 4.47, 4.61 (2H, ABq, $J_{AB}$ 12.3 Hz, $PhCH_2$), 4.63, 4.79 (2H, ABq, $J_{AB}$ 12.1 Hz, $PhCH_2$), 4.73 (1H, d, H-1), 4.83, 4.99 (2H, ABq, $J_{AB}$ 10.8 Hz, $PhCH_2$), 7.12–7.38 (20H, m, ArH), 9.77 (1H, t, *J* 1.3 Hz, HC=O); $\delta_C$ ($CDCl_3$) 22.1, 40.7, 66.9, 68.4, 73.3, 73.4, 75.1, 75.7 (8×t, 4×$PhCH_2$, $O\underline{C}H_2\underline{C}H_2\underline{C}H_2$, C-6), 70.3, 77.6, 80.0, 82.0 (4×d, C-2, C-3, C-4, C-5), 97.1 (d, C-1), 127.6, 127.7, 127.7, 127.9, 127.9, 128.0, 128.0, 128.3, 128.4, 128.4 (10×d, 20×ArC), 137.8, 138.1, 138.2, 138.8 (4×s, 4×ArC), 202.0 (d, $CH_2\underline{C}HO$); *m*/*z* ($APCI^+$) 633 ($MNa^+$, 74), 113 (100%); HRMS calcd for $C_{38}H_{46}O_7N$ ($MNH_4^+$): 628.3274; found: 628.3279.

### 3.6. 2-(N-(4-(2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyloxy)butyl)amino)benzamide **7a**

Aldehyde **6a** (120 mg, 0.29 mmol) and anthranilamide (39 mg, 0.29 mmol) were dissolved in DCE (5 ml) and sodium triacetoxyborohydride (85 mg, 0.40 mmol) was added. The mixture was stirred at room temperature for 4 h, at which point TLC (ethyl acetate:petrol, 3:2) showed the formation of a major product ($R_f$ 0.2) and the absence of any starting material ($R_f$ 0.4). The reaction mixture was partitioned between ether (100 ml) and water (100 ml) and the aqueous layer was re-extracted with ether (100 ml). The combined organic layers were washed with brine (50 ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate:petrol:triethylamine, 3:2:0.25) to afford **7a** (125 mg, 80%) as a colourless oil; $[\alpha]_D^{21}$ +85 (*c*, 1.1 in $CHCl_3$); $\nu_{max}$ (thin film) 3475 (NH), 3359 (NH), 1749 ($OCOCH_3$), 1657 ($CONH_2$) $cm^{-1}$; $\delta_H$ (400 MHz, $CDCl_3$) 1.68–1.79 (4H, m, $OCH_2C\underline{H}_2C\underline{H}_2CH_2$), 2.01, 2.03, 2.04, 2.09 (12H, 4×s, 4×$CH_3$), 3.22–3.23 (2H, m, $OCH_2CH_2CH_2C\underline{H}_2$), 3.47–3.50 (1H, m, $OC\underline{H}_aH_bCH_2CH_2CH_2$), 3.73–3.77 (1H, m, $OCH_a\underline{H}_bCH_2CH_2CH_2$), 4.01 (1H, ddd, $J_{4,5}$ 10.2 Hz, $J_{5,6}$ 2.3 Hz, $J_{5,6'}$ 4.4 Hz, H-5), 4.08 (1H, dd, $J_{6,6'}$ 12.3 Hz, H-6), 4.24 (1H, dd, H-6′), 4.87 (1H, dd, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 10.2 Hz, H-2), 5.06 (1H, at, *J* 9.7 Hz, H-4), 5.07 (1H, d, H-1), 5.48 (1H, at, H-3), 6.57–7.41 (4H, m, 4×ArH); $\delta_C$ ($CDCl_3$) 20.7 (q, 4×$CH_3$), 25.7, 27.0, 42.6, 61.9, 68.5 (5×t, $O\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2$, C-6), 67.1, 68.5, 70.2, 70.8 (4×d, C-2, C-3, C-4, C-5), 95.8 (d, C-1), 111.9, 114.5, 128.3, 133.6 (4×d, 4×ArC), 112.9, 150.1 (2×s, 2×ArC), 169.7, 170.2, 170.3, 170.7, 172.1 (5×s, 4×$\underline{C}OCH_3$, $CONH_2$); *m*/*z* ($APCI^+$) 595 ($MK^+H_2O$, 21), 561 ($MNa^+$, 45), 539 ($MH^+$, 100%); HRMS calcd for $C_{25}H_{35}O_{11}N_2$ ($MH^+$): 539.2241; found: 539.2241.

### 3.7. 2-(N-(4-(2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyloxy)butyl)amino)benzamide **7b**

Aldehyde **6b** (200 mg, 0.33 mmol) and anthranilamide (45 mg, 0.33 mmol) were dissolved in DCE (5 ml). Sodium triacetoxyborohydride (97 mg, 0.46 mmol) was added and the mixture stirred for 6 h, at which point TLC (ethyl acetate:petrol:triethylamine, 3:2:0.25) showed the formation of a major product ($R_f$ 0.3) and the absence of any starting material ($R_f$ 0.5). The reaction mixture was stirred with sodium hydrogen carbonate (5 ml of a saturated aqueous solution) for 15 min and then partitioned between ether (100 ml) and water (100 ml). The aqueous layer was re-extracted with ether (100 ml) and the combined organic extracts were washed with brine (50 ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate:petrol:triethylamine, 3:2:0.25) to afford **7b** (209 mg, 87%) as a colourless oil; $[\alpha]_D^{21}$ +36 (*c*, 1.0 in $CHCl_3$); $\nu_{max}$ (thin film) 3341 (NH), 1652 (C=O) $cm^{-1}$; $\delta_H$ (400 MHz, $CDCl_3$) 1.70–1.80 (4H, m, $OCH_2C\underline{H}_2C\underline{H}_2CH_2$), 3.19–3.21 (2H, m, $OCH_2CH_2CH_2C\underline{H}_2$), 3.44–3.48 (1H, m, $OC\underline{H}_aH_bCH_2CH_2CH_2$), 3.57 (1H, dd, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.8 Hz, H-2), 3.60–3.74 (4H, m, $OCH_a\underline{H}_bCH_2CH_2CH_2$, H-4, H-6, H-6′), 3.76–3.79 (1H, m, H-5), 3.99 (1H, at, *J* 9.2 Hz, H-3), 4.47, 4.62 (2H, ABq, $J_{AB}$ 12.3 Hz, $PhCH_2$), 4.48, 4.84 (2H, ABq, $J_{AB}$ 10.8 Hz, $PhCH_2$), 4.65, 4.79 (2H, ABq, $J_{AB}$ 12.3 Hz, $PhCH_2$), 4.77 (1H, d, H-1), 4.82, 5.00 (2H, ABq, $J_{AB}$ 10.8 Hz, $PhCH_2$), 5.73 (2H, br, s, $NH_2$), 6.57 (1H, at, *J* 7.6 Hz, ArH), 6.72 (1H, d, *J* 8.6 Hz, ArH), 7.14–7.38 (22H, m, ArH); $\delta_C$ ($CDCl_3$) 25.9, 27.0, 42.8, 67.8, 68.5, 73.2, 73.5, 75.1, 75.7 (9×t, C-6, 4×$PhCH_2$, $O\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2$), 70.2, 77.7, 80.1, 82.0 (4×d, C-2, C-3, C-4, C-5), 97.0 (d, C-1), 127.5, 127.7, 127.8, 127.9, 128.0, 128.3, 128.3, 128.4, 133.5 (9×d, 24×ArC), 137.9, 138.2, 138.3, 138.9 (4×s, 6×ArC), 172.1 (s, $CONH_2$); *m*/*z* ($APCI^+$) 754 ($MNa^+$, 88), 732 ($MH^+$, 100%); anal. found: C, 74.01; H, 6.95; N, 3.96; $C_{45}H_{50}O_7N_2$ requires: C, 73.95; H, 6.90; N, 3.83%.

### 3.8. 2-(N-(4-(α-D-Glucopyranosyloxy)butyl)amino)benzamide **8**

#### 3.8.1. Method 1

Compound **7a** (60 mg, 0.11 mmol) was dissolved in methanol (3 ml), a solution of sodium (20 mg, 0.87 mmol) in methanol (2 ml) was added and the mixture stirred. After 4 h, TLC (CMAW) indicated the formation of a major product ($R_f$ 0.4) and the absence of starting material ($R_f$ 1.0). The mixture was concentrated in vacuo and the residue purified by flash column chromatography (CMAW) to afford **8** (26 mg, 63%) as a colourless oil; $[\alpha]_D^{26}$ +57 (*c*, 0.7 in MeOH); $\nu_{max}$ (thin film) 3420 (br, OH), 1660 (C=O) $cm^{-1}$; $\delta_H$ (400 MHz, $D_2O$) 1.56–1.66 (4H, m, $OCH_2C\underline{H}_2C\underline{H}_2CH_2$), 3.07–3.20 (2H, m, $OCH_2CH_2CH_2C\underline{H}_2$), 3.26 (1H, at, *J* 9.6 Hz, H-4), 3.39–3.44 (1H, m, $OC\underline{H}_aH_bCH_2CH_2CH_2$), 3.40 (1H, dd, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.9 Hz, H-2), 3.48–3.53 (1H, m, H-5), 3.55 (1H, at, H-3), 3.58–3.64 (1H, m, $OCH_a\underline{H}_bCH_2CH_2CH_2$), 3.60 (1H, dd, $J_{5,6}$ 5.3 Hz, $J_{6,6'}$ 12.4 Hz, H-6), 3.68 (1H, dd, $J_{5,6'}$ 2.4 Hz, H-6′), 4.76 (1H, d, H-1), 6.89 (1H, at, *J* 7.6 Hz, ArH), 6.94 (1H, d, *J* 8.2 Hz, ArH), 7.39 (1H, at, *J* 7.4 Hz, ArH), 7.52 (1H, d, *J* 7.8 Hz); $\delta_C$ ($D_2O$) 21.3, 24.8 (2×t, $OCH_2\underline{C}H_2\underline{C}H_2CH_2$), 45.6 (t, $OCH_2CH_2CH_2\underline{C}H_2$), 60.8 (t, $O\underline{C}H_2CH_2CH_2CH_2$), 69.9 (d, C-4), 71.6 (d, C-2), 72.1 (d, C-5), 73.4 (d, C-3), 98.4 (d, C-1), 116.5, 120.4, 129.5, 134.1 (4×d, ArC), 145.6 (s, ArC), 173.7 (s, $CONH_2$); *m*/*z* ($APCI^+$) 393 ($MNa^+$, 54), 371 ($MH^+$, 100%); HRMS calcd for $C_{17}H_{27}O_7N_2$ ($MH^+$): 371.1818; found: 371.1811.

#### 3.8.2. Method 2

Compound **7b** (250 mg, 0.34 mmol), 10% palladium on carbon (300 mg, 0.28 mmol), and ammonium formate (324 mg, 5.1 mmol) were stirred together in methanol (5 ml) and the reaction mixture heated to 65°C. After 24 h, TLC (CMAW) showed the formation a major product ($R_f$ 0.4). The reaction mixture was cooled, filtered, concentrated in vacuo and the residue purified by flash column chromatography (CMAW) to afford **3** (96 mg, 76%) as a colourless oil identical to the material described above.

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