Biocatalytic Transfer of Pseudaminic Acid (Pse5Ac7Ac) using Promiscuous Sialyltransferases in a Chemoenzymatic Approach to Pse5Ac7Ac Containing Glycosides

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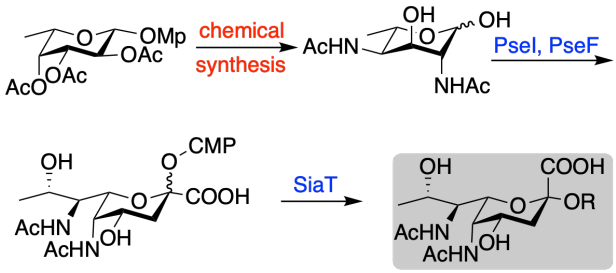
KEYWORDS: Chemo-enzymatic synthesis, Pseudaminic acid glycosides, Sialyltransferases, Sialic acid mimics, Biocatalysis

ABSTRACT: Pseudaminic acid (Pse5Ac7Ac) is a non-mammalian sugar present on the cell surface of a number of bacteria including *Pseudomonas aeruginosa*, *Campylobacter jejuni* and *Acinetobacter baumannii*. However, the role Pse5Ac7Ac plays in host-pathogen interactions remains underexplored, particularly compared to its ubiquitous sialic acid analogue Neu5Ac. This is primarily due to a lack of access to difficult to prepare Pse5Ac7Ac glycosides. Herein we decribe the *in vitro* biocatalytic transfer of an activated Pse5Ac7Ac donor onto glycosyl acceptors enabling the enzymatic synthesis of Pse5Ac7Ac containing glycosides. In a chemoenzymatic approach, chemical synthesis initially afforded access to a late stage Pse5Ac7Ac biosynthetic intermediate, which was subsequently converted to the desired CMP-glycosyl donor in a one-pot-two-enzyme process using biosynthetic enzymes. Finally screening a library of 13 sialyltransferases (SiaT) with the unnatural substrate enabled the identification of a promiscuous inverting SiaT capable of turnover to afford β-Pse5Ac7Ac terminated glycosides.



INTRODUCTION

Nonulosonic acids (NulOs) are nine-carbon α-keto-acid sugars that are ubiquitous in nature, occurring in several cell surface glycoconjugates where they play a crucial role in cell-cell interactions.[1-4] One nonulosonic acid of particular interest is non-eukaryotic α-5,7-diacetamido-3,5,7,9-tetradeoxy-L-glycero-L-manno-non-2-ulosonic acid, or pseudaminic acid (Pse5Ac7Ac **1**), and its derivatives.[5] Upon its discovery within the lipopolysaccharide *O*-antigen of *Pseudomonas aeruginosa* and *Shigella boydii* in the mid-1980s,[6] it was noted that Pse5Ac7Ac **1** possesses structural similarities to the widely prevalent *N*-acetyl neuraminic acid (Neu5Ac)[7] **2** (Figure 1, which has D-glycero-D-galacto stereochemistry).[8] A number of derivatives differentially substituted at the N5 or N7 positions of the Pse backbone have subsequently been identified in a range of glycoconjugates attached through both axial and equatorial glycosidic linkages,[9-11] in several multidrug resistant pathogens[5] where they play a crucial role in virulence.[5, 12-14]

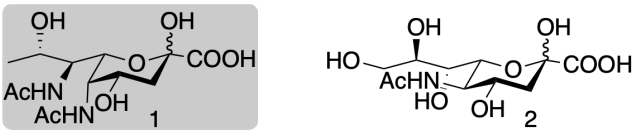
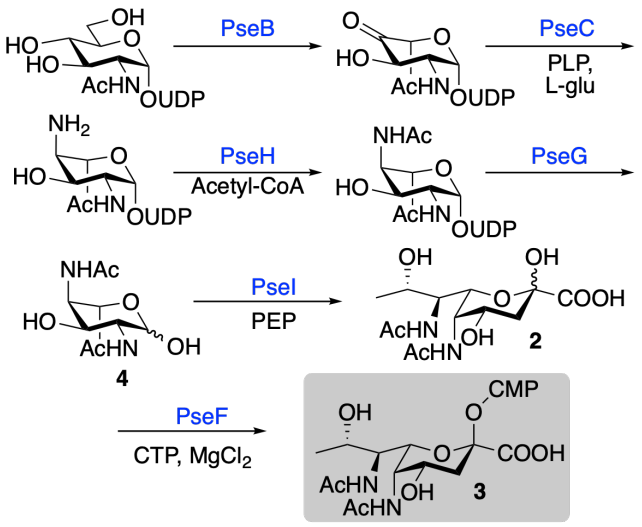


Figure 1 Nonulosonic acid structures; the pseudaminic acid Pse5Ac7Ac **1**, and sialic acid Neu5Ac **2**.

Neu5Ac **2** often exists as the terminal sugar on human cell surface glycoconjugates, and is therefore an accessible binding site for invaders, such as the influenza virus,[15] but also for interactions with host proteins, specifically Siglecs which are lectins primarily present on host immune cells.[16] Siglecs play an integral role in immune tolerance and help the immune system to recognise Neu5Ac and interpret its presence on the cell as a sign of "self",[17] a mechanism which can be exploited by bacteria able to camouflage themselves in surface Neu5Ac and evade immune detection in an example of "molecular mimicry".[18] Despite its structural similarity to Neu5Ac **2** and



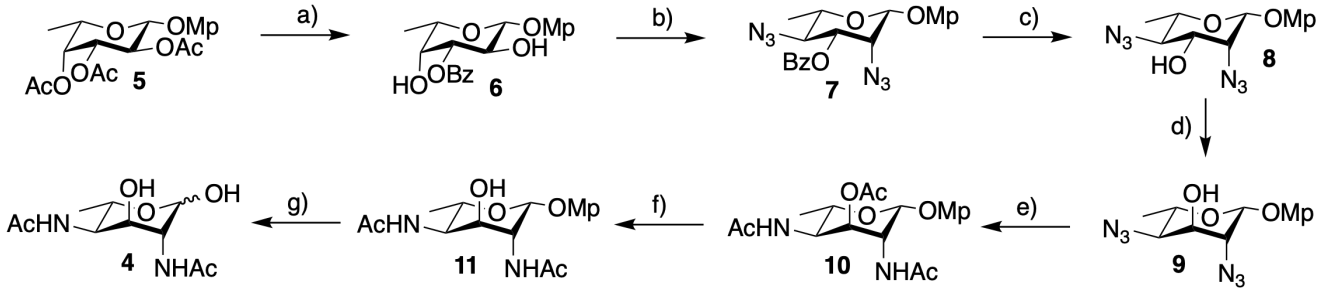
Scheme 1 Biosynthesis of CMP-Pse5Ac7Ac **3** *via* the key hexose intermediate 6-deoxy-L-AltdiNAc **4**. PLP = pyridoxal 5'-phosphate; L-Glu = L-glutamate; PEP = phosphoenolpyruvate; CTP = cytidine monophosphate.

aforementioned presence on pathogenic bacterial cell surfaces, there are precious few studies[19] exploring how Pse5Ac7Ac **1** might bind to host proteins compared to Neu5Ac **2**, and therefore if bacteria employ Pse glycans to aid immune evasion. Such studies have been hindered by a lack of access to Pse-glycosides, specifically those which mimic natural Neu5Ac capped glycoconjugates and would provide a means to compare the effects of a terminal Neu5Ac or Pse5Ac7Ac architecture on glycan-receptor interactions. This is because production of Pse glycosides by purely chemical methods is rare, and although impressively elegant, all reported strategies to date have been multi-step resulting in a low overall yield, and face additional challenges in controlling the stereochemistry of Pse-glycosidic linkages during glycosylation.[20-27] Conversely, the highly stereo- and regioselective nature of enzymatic glycosylation make it an attractive route to Pse-glycosides. *In vivo* studies have tentatively identified glycosyltransferases proposed to utilise CMP-Pse donors (pseudaminyltransferases) in a range of bacteria.[28, 29] However, currently no direct *in vitro* enzymatic transfer of CMP-Pse using a glycosyltransferase has been reported. Furthermore the sole *in vitro* enzymatic synthesis of CMP-Pse5Ac7Ac **3** in the literature is prohibitively expensive requiring both costly UDP-GlcNAc starting material and an acetyl-CoA cofactor in the early steps in the pathway (Scheme 1).[30]

Therefore to facilitate future comparisons of the effects of Neu5Ac and Pse5Ac7Ac capped glycoconjugates on protein binding interactions, herein we address the lack of practical procedures to Pse-glycosides through the union of both chemical and enzymatic synthesis. Concluding with the transfer of CMP-Pse5Ac7Ac **3** onto a range of glycosyl acceptors exploiting a promiscuous sialyltransferase to afford β-2,3/2,6-linked Pse di- and trisaccharides mimics. We initially targeted the chemical synthesis of 6-deoxy-L-AltdiNAc **4** (Scheme 1), a biosynthetic precursor of Pse5Ac7Ac **1**, and a substrate for PseI, a Pse5Ac7Ac synthase.[12, 31] Our aim was to utilise PseI in combination with PseF, a CMP-Pse5Ac7Ac acid synthetase,[30] in a one-pot procedure to synthesise CMP-Pse5Ac7Ac **3** from the reducing sugar **4**. The CMP-sugar **3** could then be used as a potential glycosyl donor in a sialyltransferase (SiaTs) activity screen, with the aim of identifying promiscuous enzymes capable of synthesising Pse containing glycosides.

RESULTS AND DISCUSSION

Chemical synthesis of 6-deoxy-L-AltdiNAc 4. We established an efficient chemical route to 6-deoxy-L-AltdiNAc **4** starting from the readily synthesised L-fucoside 5 (Scheme 2).[32] The synthesis required a triple inversion of stereochemistry at the 2, 3, and 4 positions, with the order in which these inversions were performed proving crucial to success. Following global deacetylation of **5**, regioselective *O*-3 benzoylation was achieved with diphenyl borinate to afford the L-fucosyl 2,4-diol **6**. Treatment of **6** with Tf_2O in pyridine yielded a 2,4-bis-triflate, which was then treated with NaN_3 to access the rare deoxy-amino-L-mannose derivative **7** *via* double, parallel inversions.[33] Notably when double inversions were attempted on the *O*-3 benzoyl epimer of **6**, only decomposition was observed, presumably due to the axial benzoyl group increasing steric hindrance during equatorial attack of *O*-4, as previously also noted by Ito and co-workers.[24] Debenzoylation afforded compound **8**, which was then trifylated at *O*-3 to produce an intermediate triflate, and once more subjected to inversion without purification through treatment with $TBANO_2$. The reaction proceeded cleanly and 2,4-di-azido altropyronaside **9** was isolated in 46% yield, over two steps. However, we noted that if the α-anomer of **8** was treated under similar conditions, or subjected to oxidation and subsequent hydride reduction, no inversion of steroechemistry at *O*-3 was observed in our hands, likely a result of unfavourable 1,3-diaxial in teraction experienced during axial attack upon *O*-3. Staudinger reduction of the 2,4-di-azido altropyronaside **9** with PMe_3, followed



Scheme 2 Chemical synthesis of 6-deoxy-L-AltdiNAc **4**. *a)* i) NaOMe, MeOH. ii) Diphenyl borinate, DIPEA, BzCl, CH_3CN, 80% over 2-steps. *b)* i) Tf_2O, Pyridine, DCM, 0 °C. ii) NaN_3, DMF, 110 °C, 58% over 2-steps. *c)* NaOMe, MeOH, 95%. *d)* i) Tf_2O, Pyridine, DCM, 0 °C. ii) $TBANO_2$, CH_3CN, 70 °C, 46% over 2-steps. *e)* i) PMe_3, NaOH, THF, 60 °C. ii) Ac_2O, Pyridine, 69% over 2-steps. *f)* NaOMe, MeOH, 81%. *g)* CAN, CH_3CN:H_2O (4:1 v/v), 94%, α:β 1:3.

by acetylation, afforded compound **10**, which was then selectively *O*-deacetylated to afford 2,4-di-acetamido altropyronaside **11**. Ceric ammonium nitrate assisted deprotection of the anomeric methoxyphenyl group finally yielded the desired 6-deoxy-AltdiNAc reducing sugar **4** in an 11% overall yield.

Enzymatic production of CMP-Pse5Ac7Ac 3. Synthesis of the desired CMP-Pse5Ac7Ac **3** nucleotide donor was achieved utilizing the final two enzymes from the biosynthetic pathway; PseI and PseF (Figure 2). *C. jejuni* PseI was initially expressed and purified under optimized conditions affording 20 mg L^{-1} (Figure 2a).[31] Sequence alignment with *H. pylori* PseF[34] revealed a putative CMP-Pse5Ac7Ac synthetase gene present in the gram-negative *Aeromonas caviae* genome.[28] Subsequent expression of *A. caviae* PseF was optimised and resulted in the purification of soluble enzyme affording 13 mg L^{-1} (Figure 2a). Activity of the Pse5Ac7Ac synthase PseI was monitored by negative ion electrospray ionization (ESI) LC-MS during incubation with 6-deoxy-L-AltdiNAc **4** at pH 7.4 with a small excess of the required phosphoenol pyruvate co-factor.[31] A peak associated with production of Pse5Ac7Ac **1** ($[M-H]^-$ 333) was immediately observed without addition of exogenous divalent metal ions (Figure S2). Upon addition of *A. caviae* PseF to the reaction mixture with excess CTP and $MgCl_2$ a peak associated with the production of CMP-Pse5Ac7Ac **3** ($[M-H]^-$ 638) was observed. Hence demonstrating that CMP-Pse5Ac7Ac **3** can be enzymatically produced in one-pot from chemically synthesized 6-deoxy-L-AltdiNAc **4** (Figure 2c). The reaction was stopped after four hours and the product purified by size exclusion chromatography (Bio-Gel® P-2 resin Biorad), NMR and LC-MS characterization of the enzymatic product by comparison to previously published data confirmed the synthesis of αCMP-Pse5Ac7Ac **3** (Figure S25-26).[30] Calculation of the difference in chemical shift between the $H3_{ax}$ and $H3_{eq}$ (Δppm $H3_{eq}$−$H3_{ax}$ = 0.62 ppm) was indicative of the axially orientated *O*-CMP group (Figure 3).[35, 36]

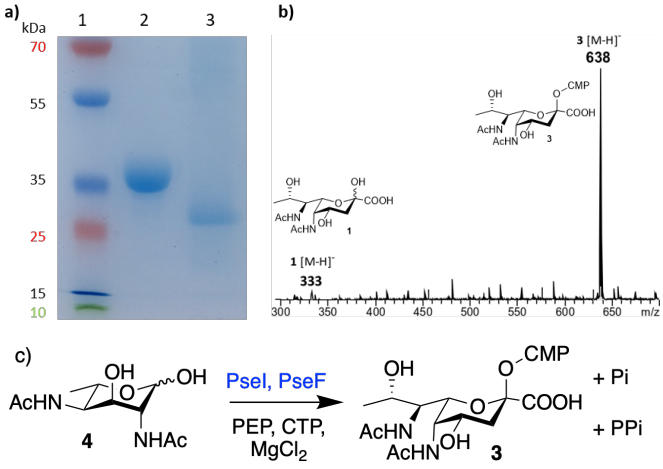


Figure 2 a) 12% SDS PAGE analysis of purified enzymes; Lane 1: Molecular weight ruler, Lane 2: *C. jejuni* PseI (40.8 kDa), Lane 3: *A. caviae* PseF (28.0 kDa) **b)** Negative ESI LC-MS for the one-pot, two enzyme synthesis of CMP-Pse5Ac7Ac **3** from 6-deoxy-AltdiNAc **4** **c)** Use of the chemically derived 6-deoxy-L-AltdiNAc 4 in the one-pot enzymatic synthesis of CMP-Pse5Ac7Ac **3**.

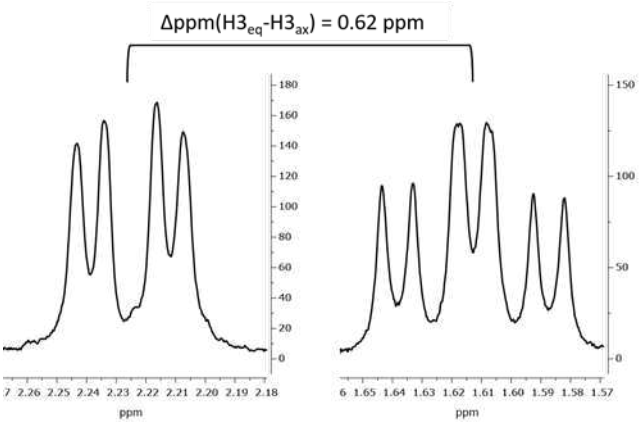
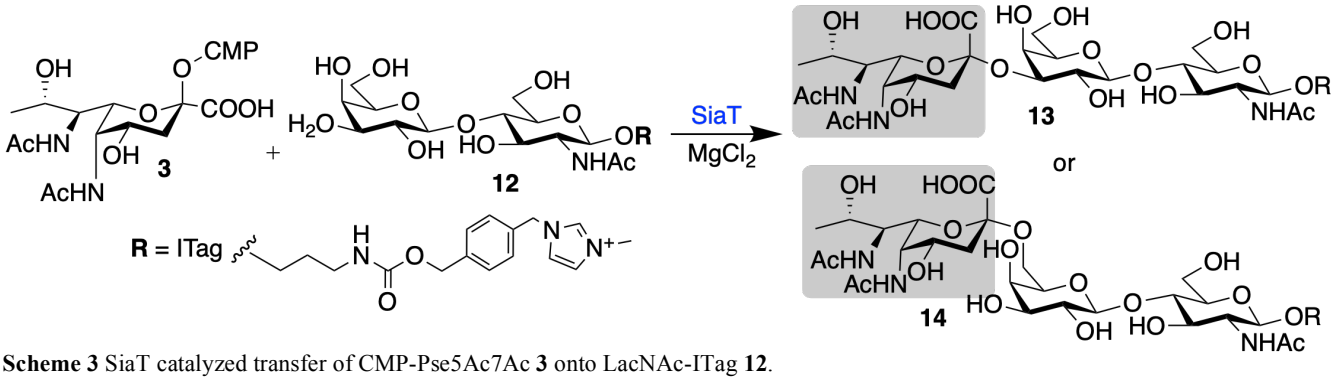


Figure 3 The chemical shift of the H3eq (2.23 ppm) and H3ax (1.61 ppm) proton NMR peaks used to assign the PseF enzymatic product as αCMP-Pse5Ac7Ac **3**.

Sialyltransferase activity screen using α-CMP-Pse5Ac7Ac 3. *In vitro* studies of well-characterised sialyltransferases (SiaTs) have shown that a number of enzymes have promiscuity towards both the donors and acceptors they can turnover. A range of natural and bioorthogonal tagged CMP-Neu5Ac derivatives and other CMP-nonulosonic acids (such as CMP-Leg5Ac7Ac) have previously been utilised as donors.[6, 35, 37-38] Pse5Ac7Ac **1** differs from Neu5Ac **2** functionally; C7 has an acetamido and C9 loses a hydroxyl, and stereochemically; C5, C7 and C8 are epimeric. However SiaT promiscuity had previously been observed with sugars that include some of these modifications, C9 deoxy[37] and C7 *N*-acyl[38] sugars for example, therefore it was reasoned that these enzymes may also be functional in the chemoenzymatic synthesis of Pse5Ac7Ac-based glycosides. A library of 13 bacterial SiaTs were therefore screened for their ability to utilise αCMP-Pse5Ac7Ac **3** as a glycosyl donor, including the three enzymes that had previously shown activity towards CMP-Leg5Ac7Ac.[37, 39] A range of characterised and putatively assigned SiaTs were considered (Table S1), from three different GT families; GT42, GT52 and GT80, with an imidazolium tagged-*N*-acetyllactosamine (LacNAc-ITag)[40] **12** acceptor (Scheme 3). Two SiaTs chosen had previously been shown to catalyse the glycosylation of CMP-Neu5Ac and a LacNAc derivative labelled with a *N*-benzenesulfonyl-type ITag,[41] which bears a similar imidazolium label to LacNac-ITag **12**. The use of the cationic ITag label decreases the mass spectrometry detection limit for observing reaction components and increases the spectral peak intensity of LacNAc-ITag containing moieties, thus facilitating the screening of enzyme activity.[42-44]



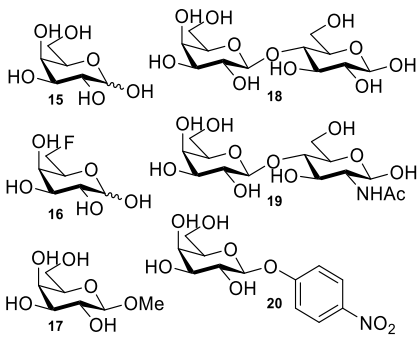
Scheme 3 SiaT catalyzed transfer of CMP-Pse5Ac7Ac **3** onto LacNAc-ITag **12**.

Reaction conditions were identical for all SiaTs; incubating a 1:4 ratio of donor to acceptor at pH 7.5, for 18 hours. Analysis of ITag containing compounds with MALDI-ToF showed starting material remained in all reactions but an additional peak was present with four enzymes (all GT80), which was assigned to the desired Pse5Ac7Ac-LacNAc-ITag (m/z 985.4) (Table S1, Figure S3). Based on the reported enzymatic characterisation of these enzymes, under these reaction conditions it was predicted that the *Photobacterium sp. JT-ISH-224*[45]; *Pasteurella dagmatis*[46] and truncated *Pasteurella multocida*[38] SiaTs would all catalyse production of the β-2,3-linked glycoside **13** and the *Photobacterium leiognathi*[47] SiaT would result in a β-2,6-linked glycoside **14**. Not all SiaTs tested herein have previously been characterised and hence negative hits are to be treated with caution as pseudo-pseudaminyltransferase activity may also be possible under alternate conditions.[38]

To corroborate the MALDI-TOF results for the positive hits, the same reactions were analysed using negative ESI LC-MS (Figure S4), and in all cases no remaining CMP-Pse5Ac7Ac **3** starting material was detected. Considering the *Pasteurella multocida* SiaT (tPm0188Ph) affords β-2,3-linked glycosides at pH 7.5, but can also potentially be tuned to produce α2-6-glycosides under different conditions,[38] it was chosen for further investigation. Specifically, αCMP-Pse5Ac7Ac **3** was screened with a range of galactose acceptors **15-20**, in a 1:4 donor to acceptor ratio (Table 1) to profile tPm0188Ph acceptor promiscuity. Negative ESI UPLC-MS standard curves for Pse5Ac7Ac **1** and αCMP-Pse5Ac7Ac **3** at known concentrations (Figure S5) were used to calculate the level of conversion to the glycoside product by determining the amount of unreacted material present. In 4 h experiments high levels of conversion (>75%) were achieved with five of the galactose-based acceptors **15**, **17-20** (Table 1). Similar to results previously obtained with the CMP-Neu5Ac donor, Lactose **18** and LacNAc **19** were accepted particularly well by this enzyme (90% and 91% conversion respectively),[38] as was Galβ-*p*NP **20** (91% conversion). This was significantly higher than with monosaccharides **15** and **17**, presumably as **18-20** more closely mimic the structure of the native disaccharide acceptor.[48] A 57% conversion was also observed with the unnatural 6F-galactose **16** demonstrating that under the reaction conditions screened the *P. multocida* enzyme is indeed likely forming a 2,3-linkage. It was proposed that the reduction of activity with 6F-galactose **16** could be attributed to a loss of hydrogen bonding to Asn85 that is predicted in the galactose binding region with the native C6-OH.[48] A time-course experiment using LacNAc acceptor **19** demonstrated that the majority of the αCMP-Pse5Ac7Ac **3** donor was turned over to glycoside product within 1 h (Figure S10), and although some formation of hydrolysis product Pse5Ac7Ac **1** was observed by negative ion LCMS, the relative proportion did not change over the course of the reaction (0-4 h) suggesting minimal enzymatic hydrolysis of the donor.

Table 1 *Pasteurella multocida* pseudo-pseudaminyltransferase (tPm0188Ph) catalyzed transfer of α-CMP-Pse5Ac7Ac **3** onto galactose-based acceptors (**15-20**).

Acceptor	Conversion
15	78%
16	57%
17	80%
18	90%
19	91%
20	91%



Docking[49] of αCMP-Pse5Ac7Ac **3** into the *P. multocida* crystal structure[48] showed that this donor could be accepted in a conformation analogous to that reported for bound CMP-3F(a)Neu5Ac (Figure 4.a) reinforcing the donor promiscuity observed experimentally. Previous donor-bound crystal structures highlight that the majority of interactions occur with the nucleotide moiety which is buried within the enzyme active site,[48] docking of αCMP-Pse5Ac7Ac **3** does not disturb any of these interactions, despite a slight perturbation of the phosphate group (Figure S7). Importantly when αCMP-Pse5Ac7Ac **3** is docked in its lowest energy conformation with the CMP moiety occupying this site, there is no steric clashes between the Pse5Ac7Ac sugar moiety and the enzyme (Figure 4.b). Similarly, the binding affinity of docked αCMP-Pse5Ac7Ac **3** (-10 kcal mol^{-1}) was only marginally lower than docked βCMP-Neu5Ac (-10.2 kcal mol^{-1}) suggesting that any loss of binding interactions due to structural differences are compensated for by other interactions. However, it is notable that in a direct competition experiment between the βCMP-Neu5Ac and αCMP-Pse5Ac7Ac **3** donors in the presence of LacNAc acceptor **19** formation of 10-fold greater Neu5Ac-LacNAc over Pse5Ac7Ac-LacNAc was observed (Figure S8), indicating that *in vitro* the *P. multocida* enzyme has an established preference for the Neu5Ac scaffold.

Co-crystallisation with CMP-3F(a)Neu5Ac previously identified five hydrogen bonds (two water-mediated) and one ion pair interaction between the Neu5Ac moiety and enzyme.[48] Analysis of αCMP-Pse5Ac7Ac docked into tPm0188 shows that there are also five hydrogen bonds (one water-mediated) and one ion pair interaction possible, albeit to different resi-

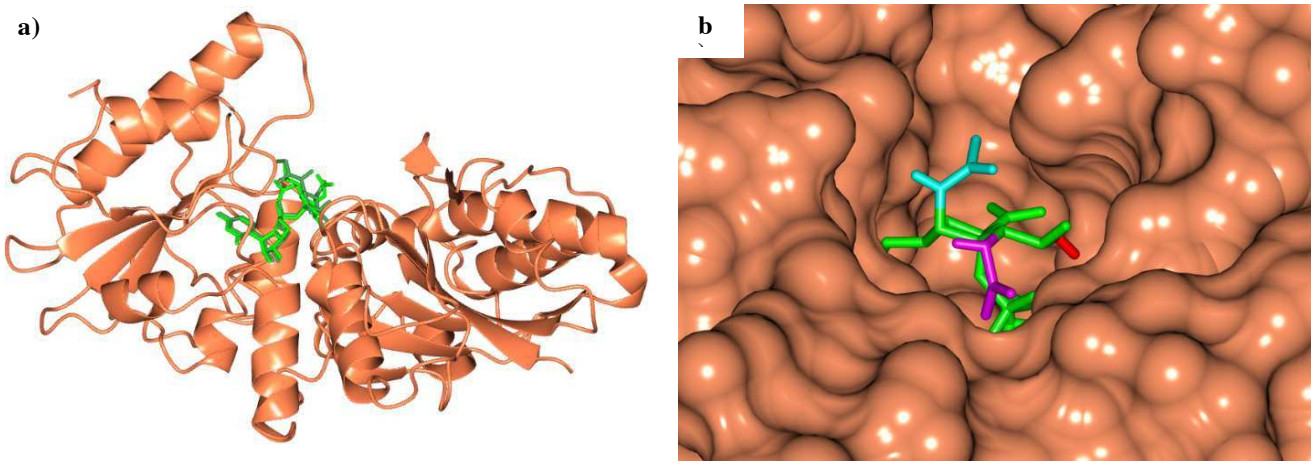


Figure 4 αCMP-Pse5Ac7Ac **3** (green cylinder model) docked into *Pasteurella multocida* tPm0188Ph (coral) **a)** overlaid with CMP-Neu5Ac (lawn green cylinder model) to show the heavily conserved binding motif, and **b)** a tPm0188Ph space filling model (coral) to show the orientation of the epimeric Pse5Ac7Ac sugar ring substituents (C5 acetamido-cyan, C7 acetamido-magenta, C8 hydroxyl-red).

dues in the active site in some cases (Figure 5). The C2 carboxyl is orientated to allow for ion pair interactions with Arg63 in both Neu5Ac and Pse5Ac7Ac, similarly the C4 hydroxyl in both molecules can form an H-bond with the Asp141 carbonyl backbone. The Trp270 side chain forms a H bond with both Neu5Ac and Pse5Ac7Ac moieties, the C7 hydroxyl and C5 acetamido carbonyl respectively, which occupy a similar space due to the opposing stereochemistry at these two centres. In Pse5Ac7Ac, the C5 acetamido group forms a second H bond with the amine proton interacting with the Asp141 side chain carbonyl in this configuration, whereas this H bond is to Ser143 in Neu5Ac. The C8 hydroxyl in both molecules form H bonds, a water mediated bond occurs with Thr267 in Neu5Ac, however in Pse5Ac7Ac the C8 hydroxyl can directly interact with His311 due being epimerised at this position. Finally, the Pse5Ac7Ac C7 acetamido group has a water mediated H bond between the carbonyl moiety and the backbone carbonyl of Ala354.

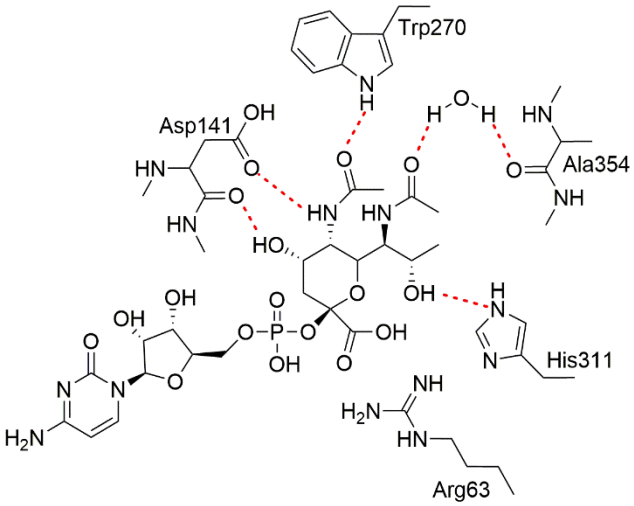
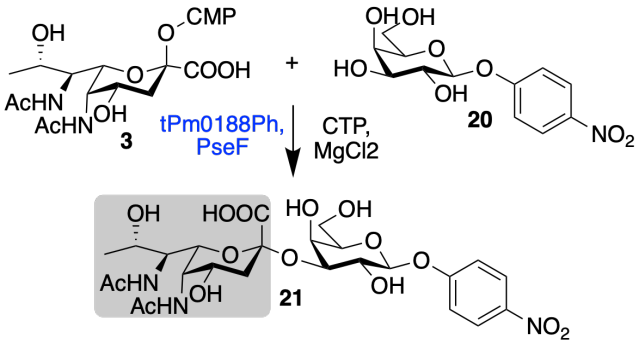


Figure 5 Binding interactions between the Pse5Ac7Ac moiety and *Pasteurella mutocida* tPm0188Ph when αCMP-Pse5Ac7Ac **3** is docked *in silico* into the active site.

tPm0188Ph catalyzed synthesis of Pse5Ac7Acβ-2,3-Galβ-*p*NP 21. The *P. multocida* enzyme (tPm0188Ph) has previously been shown to act as an inverting SiaT transferring βCMP-Neu5Ac to galactosides to preferentially make an α-2,3-linkage at pH 7.5-9.0 or an α-2,6-linkage at pH 5.0-5.5.[38] Therefore a β-2,3-Pse5Ac7Ac-galactoside would be the predicted product when using tPm0188Ph with αCMP-Pse5Ac7Ac **3** under the reaction conditions employed (pH 7.5) (Scheme 4). However further characterisation of the pseudaminyl galactoside was required to confirm the regio- and stereo- selectivity of the pseudo-pseudaminyl transferase activity. Therefore a preparative scale reaction containing αCMP-Pse5Ac7Ac **3** (9.5 mg), four equivalents of Galβ-*p*NP **20** (20.5 mg) and tPm0188Ph was used to afford enough product for purification and characterization (see SI for full details). PseF, CTP and $MgCl_2$ were also included in the reaction mixture to recycle any hydrolysed Pse5Ac7Ac **1** into αCMP-Pse5Ac7Ac **3** and drive the reaction towards the product. The reaction was stopped when after 18 hours (conversion > 90%) and following purification, NMR analysis confirmed the product as β-2,3-Pse5Ac7Ac-Galβ-*p*NP **21** (Figure S27-28), formed in a 2.1 mg (23 %) yield. ^{13}C NMR comparison of the Galβ-*p*NP **20** and product **21** revealed a downfield chemical shift of the galactose C3 by 2.65 ppm, with no significant galactose C6 chemical shift perturbation, indicative of glycosylation at the galactose C3.[38] Once again, the anomeric stereochemistry was assigned using the difference in chemical shift between the $H3_{ax}$ and $H3_{eq}$ with a value of Δppm $H3_{eq}$–$H3_{ax}$ = 0.9 ppm indicative and characteristic of a Pse5Ac7Ac β-linkage (Figure 6).[35, 36]

CONCLUSIONS

This work provides the first examples of chemoenzymatically synthesised Pse containing glycosides, which are in in-



Scheme 4 tPm0188Ph catalysed synthesis of Pse5Ac7Acβ-2,3-Galβ-*p*NP **21**.

creasingly high demand for biological studies. Given that the chemical syntheses of such molecules are particularly arduous, the use of enzymes in tandem with synthetic chemistry affords a more expedient and efficient route to the synthesis of this important class of glycans. This work also provides Pse5Ac7Ac containing mimics of Neu5Ac capped glycoconjugates, enabling comparative exploration of receptor-glycan interactions, particularly relevant for probing the interactions between bacterial surfaces and Siglec proteins integral to host immune responses. Notably, as no native pseudaminyltransferases have yet been shown to be active *in vitro*, our demonstration that the well-characterised, highly stable, sialyltransferase from *P. multocida* displays activity with α-CMP-Pse5Ac7Ac **3**, provides an impetus to study the potential turnover of Pse glycans by other sialic acid processing enzymes. Furthermore *P. multocida* tPm0188Ph and other promiscuous sialyltransferases may offer an expedient route to the synthesis of galactose linked Pse-glycosides present in the *O*-antigens of pathogenic bacteria including *enteroinvasive E. coli*,[50] and *S. boydii*[51] serotypes.

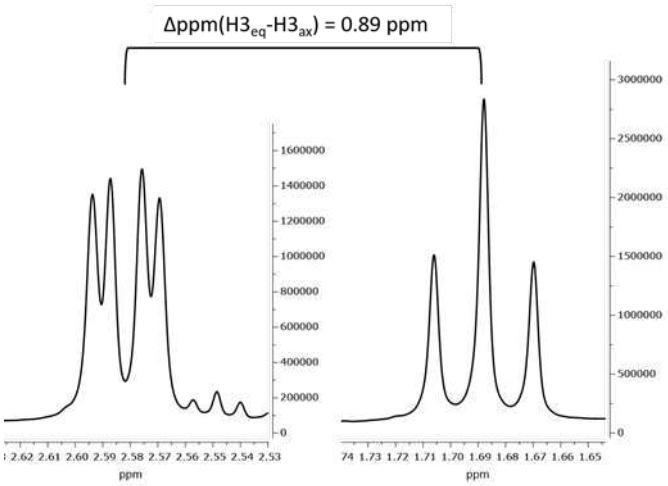


Figure 6 The chemical shift of the H3eq (2.58 ppm) and H3ax (1.69 ppm) proton NMR peaks used to assign the *P. multocida* tPm0188Ph SiaT product as Pse5Ac7Acβ-2,3-Galβ-pNP **21**.

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Author Contributions

E.K.P.F., H.S.C., T.K., K.H., P.B. performed protein production and enzymatic transformations; G.G., D.B., J.M.P., and H.L. performed chemical transformations; S.R. cloned A. caviae PseF; G.P.S., J.G.S., M.C.G., S.F., G.T., and M.A.F. supervised the project; M.A.F., E.K.P.F., and H.S.C., wrote the paper and designed the study, and all authors commented on the paper.

Funding Sources

This work was supported by The University of York, the BBSRC (BB/M02487X/1, T.K.), the EPSRC (EP/P030653/1. D.B.), and The Rosetrees Trust. MCG thank the European Research Council (ERC-COG: 648239) and BBSRC (BB/M028976/1, J.M.P and H.L).

Notes

There are no conflicts of interest to declare.

ASSOCIATED CONTENT

Supporting Information.

The supporting Information, including full experimental, assay results and NMR characterization, is available free of charge via the Internet at http://pubs.acs.org.

ACKNOWLEDGEMENTS

We thank Dr Simon Charnock of Prozomix Ltd for supplying SiaTs used in the pseudaminyltransferase activity screen, Dr. Ed Bergstrom and The York Centre of Excellence in Mass Spectrometry. The York Centre of Excellence in Mass Spectrometry was created thanks to a major capital investment through Science City York, supported by Yorkshire Forward with funds from the Northern Way Initiative, and subsequent support from EPSRC (EP/K039660/1; EP/M028127/1).

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